# THE RELATIONSHIP BETWEEN BLOOD PRESSURE, LOCOMOTOR PERFORMANCE, AND HEMORRHAGE AFTER NOXIOUS INPUT

A Dissertation

by

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# ABSTRACT

Pain input after a spinal cord injury (SCI) has been shown to be detrimental to recovery. Animal models show that noxious input soon after injury leads to an increase in hemorrhage three hours after stimulation and long-term detriments in locomotion and lesion extent. In this dissertation, I explored the relationship between noxious input and hemorrhage, and evaluated whether hypertension contributes to these effects.

The current dissertation employed a rat contusion model of SCI. The first set of experiments found that electrical stimulation increased hemorrhage when given at time points soon after injury. I also established the minimum shock duration and intensity that induces hemorrhage. The next set of experiments showed that electrical stimulation also induced an acute increase in blood pressure. Stimulation with capsaicin did not change blood pressure, but it did increase hemorrhage. Both forms of stimulation had an acute effect on locomotor performance.

In the next experiment, blood pressure and hemorrhage were examined after controllable and uncontrollable stimulation. Previous research has shown that only uncontrollable stimulation causes a change in locomotor recovery after SCI. The present study found that behavioral control affected blood pressure, but did not have a significant effect on acute hemorrhage or locomotor performance.

The next set of experiments found sex and age differences in hemorrhage and blood pressure after noxious input. Females showed the same increase in blood pressure and decrease in locomotor performance after stimulation, but did not show the increase

in hemorrhage. Females and males showed identical results after capsaicin.

The last set of experiments pharmacologically manipulated blood pressure after SCI. Decreasing systolic blood pressure with prazosin before shock prevented the induction of hemorrhage at three hours, but did not improve long-term recovery at 21 days. Increasing the blood pressure with norepinephrine after SCI decreased locomotor performance but did not increase hemorrhage.

These studies highlight the importance of blocking pain and blood pressure spikes after SCI. While blood pressure was not affected by capsaicin treatment, it did seem to correlate with hemorrhage and locomotion after stimulation. Further, blocking or inducing changes in blood pressure had effects on hemorrhage and locomotor performance, respectively.

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# CHAPTER I

# INTRODUCTION AND LITERATURE REVIEW

## Overview

Pain is meant to be adaptive. When functioning properly, pain signals to the body that there is an injury so that the person or animal can avoid further harm. After a SCI, there is often an increase in pain input due to the injury itself or associated injuries (e.g., fractures, contusions, etc.). However, after a spinal cord injury the signals to and from the brain are often damaged or disrupted. This can cause pain to become maladaptive because the brain cannot modulate spinal nociceptive circuits and quell over-excitation. Research examining how pain input after a SCI affects recovery has shown that the activation of nociceptive (pain) fibers shortly after SCI undermines locomotor performance and increases the extent of secondary injury as evidenced by an increase in lesion size and amount of hemorrhage (Turtle et al., 2017). Determining the stimuli capable of inducing these detrimental effects is important for understanding how to treat and prevent further injury after SCI. My focus will be on the expansion of the hemorrhage.

An injury to the spinal cord typically causes a shearing of blood vessels, leading to a primary hemorrhage (Tator & Koyanagi, 1997). The size of the primary hemorrhage is correlated with the severity of the injury. In some cases, a secondary hemorrhage processes can become activated due to the failed structural integrity of capillaries (Simard et al., 2007). This process is known as progressive hemorrhagic necrosis (PHN)

and is linked to the formation of the SUR1-TRPM4 complex (Simard et al., 2007). Once activated, pain leads to red blood cell infiltration into the spinal cord, which is toxic to nervous tissue, leading to poor behavioral outcomes. In our model, nociceptive stimulation (i.e., electrical stimulation or capsaicin injection) produces a significant increase in the secondary hemorrhage and diminished recovery (Turtle et al., 2017). One possible cause of the secondary hemorrhage seen after nociceptive stimulation is a change in blood pressure as a result of electrical stimulation. I hypothesized that an increase in blood pressure could cause the capillaries to fail and begin the process of PHN, accounting for the increased cell death, lesion expansion, and poor behavioral outcomes seen after nociceptive stimulation.

Upon completion of this project, I will better understand the stimulation parameters necessary to produce a secondary hemorrhage after SCI. Further, I will determine whether changes in blood pressure play a role in hemorrhage expansion, potentially providing a novel strategy for improving behavioral outcomes after SCI.

## Spinal Cord Injury

### *General Population*

There are approximately 285,000 people currently living with an SCI in the United States as of 2016, with approximately 17,000 new cases each year (NSCISC, 2016). The mean age of onset of SCI is 42 years with a greater proportion of males (80.7%). The most common level of injury is at the cervical level (54.2 %); this is followed by thoracic (35%), lumbar (10.4%) and sacral injuries (0.4%). Etiology of SCI includes vehicle crashes (38.4%), falls (30.5%), acts of violence (13.5%), and

sports/recreation activities (8.9%). Due to the etiology of injury, people with spinal cord injuries often have injuries to the vertebral column (e.g., fractures or dislocations; 79.8%) and other parts of the body (38.8%) (NSCISC, 2016).

### *Current Trends in SCI*

Among new injuries, there has been an increase in the proportion of female sustaining an SCI, from 19.7% before 1970's to 21.7 % in 2005 (DeVivo & Chen, 2011). This increase is mostly due to the increase in elderly females sustaining an injury, matching the increase in the age of general population. This has led a number of researchers to examine the effects of both sex and age after SCI (Datto, Yang, Dietrich, & Pearse, 2015).

Examination of sex has found that the cause of SCI differs between males and females, with females more likely to be injured in a vehicle accident (female, 52.2%; males, 39.9%) and males more likely to be injured in sport and violence related activities (males, 18.5 and 11.3%, respectively; females 11.4 and 5.6%, respectively) (NSCISC, 2016).

When examining sex differences in recovery after SCI in humans, researchers have found mixed results. Human studies seem to show little evidence of improved motor function. For example, one study found more improvement in females on the MSCIS motor index score when comparing hospital admission scores to their scores one year later. However, when comparing the FIM motor score, males showed better improvement between discharge and rehabilitation (Lammertse, Jackson, & Sipski, 2004). Similarly in a study comparing sex differences after comparable cervical injuries,

it was found that male and females showed similar neurological and clinical outcomes, with females actually showing an increase susceptibility to depression and deep vein thrombosis (Furlan, Krassioukov, & Fehlings, 2005).

Researchers examining sex differences in animals have observed better functional outcomes in females compared to males after SCI (Datto, Bastidas, et al., 2015; Datto, Yang, et al., 2015). In one study, female rats were found to show better gross locomotor improvements compared to males after a moderate thoracic SCI, after controlling for weight and age differences (Datto, Bastidas, et al., 2015). Female rats showed more tissue sparing at the lesion compared to males (Datto, Bastidas, et al., 2015; Fee et al., 2007). Further, locomotor improvement was found to strongly correlate with later lesion volume. Similar results were found after a mild and severe thoracic spinal cord contusion (Hauben, Mizrahi, Agranov, & Schwartz, 2002). Interestingly, sex differences are not seen in nude mice (Hauben et al., 2002).

One explanation for this difference between sexes is the differences in circulating sex hormones. There have been few human studies examining hormone therapy. One study examining progesterone co-administered with Vitamin D showed improved sensory and motor scores six months after treatment, with people receiving the first dose within four hours showing the best recovery (Aminmansour et al., 2016). Another study examining testosterone replacement therapy in chronically injured males showed improved muscle mass and energy expenditure (Bauman, La Fountaine, Cirnigliaro, Kirshblum, & Spungen, 2015). Examination of the role of sex hormones in animals has been mixed. Some studies show improved locomotor score and increase tissue sparing

after prolonged progesterone and estrogen treatment in rats (Garcia-Ovejero et al., 2014; Hu et al., 2012; Mosquera et al., 2014; Samantaray et al., 2016; Sribnick et al., 2010; Thomas, Nockels, Pan, Shaffrey, & Chopp, 1999; Zendedel et al., 2017). Others show no benefits of treatment (Fee et al., 2007). Progesterone has been shown to impact nociception, preventing allodynia associated with neuropathic pain in male rats (Coronel, Labombarda, De Nicola, & Gonzalez, 2014; Coronel, Labombarda, Villar, De Nicola, & Gonzalez, 2011; Coronel et al., 2016). Other work suggests that testosterone is detrimental to recovery in female rats and that a testosterone antagonist or castration improve recovery in male rats (Hauben et al., 2002). However, research examining testosterone treatment in rats after SCI has found that it protects against muscle and dendritic atrophy (Byers et al., 2012; Gregory, Vandenborne, Huang, Ottenweller, & Dudley, 2003). In another study testosterone transiently improved locomotor behavior (Byers et al., 2012; Wu et al., 2012; Yarrow et al., 2014).

**Polytrauma**

In humans, polytrauma after SCI is generally correlated with worse outcomes (Kushner & Alvarez, 2014)). One of the most common associated injuries is traumatic brain injury (TBI), which is seen in 24-60% of SCI cases (Macciocchi, Seel, Thompson, Byams, & Bowman, 2008; Sommer & Witkiewicz, 2004). Another common injury includes fractures to the extremities (i.e. arms and legs), which are seen in around 11% of SCI patients (Comarr, Hutchinson, & Bors, 1962). How polytrauma affects long-term recovery is often difficult to assess in humans as recovery outcomes are confounded with etiology, severity, and injury level after SCI (Macciocchi et al., 2008).

A few animal studies have examined polytrauma after SCI and found that associated injuries can lead to lower locomotor recovery (Wang et al., 2011). In one examining femoral fracture after SCI in rodents, it was found that locomotor recovery was significantly reduced up to 28 days after injury (Wang et al., 2011). The same decrease was not seen in sham subjects given a femoral fracture. Similarly, after concurrent contralateral TBI and SCI, animals showed deficits in forelimb recovery (Inoue et al., 2013). More work is needed to better understand the interaction between damage to the spinal cord and injuries to other parts of the body on recovery outcomes.

## Pain Input Affects Recovery After SCI

### *Pain in Humans*

Whether originating from the injury itself or from polytrauma, pain is often a part of SCI in both the acute and chronic phase of injury (Kennedy, Frankel, Gardner, & Nuseibeh, 1997). One study found that upon admission to the hospital, 76% of SCI patients reported the presence of pain (Cairns, Adkins, & Scott, 1996). Of those, 69% reported CESD pain scores above 65, indicating problematic high levels of pain. While the number of people suffering from high levels of pain decreases over time (43-53%), the overall number of people experiencing pain stayed the same (70-80%) (Cairns et al., 1996; Siddall, McClelland, Rutkowski, & Cousins, 2003). This early experience of severe pain has been shown in humans to correlate with the development of later pain syndromes, an increase in depression, and a decrease in perceived health (Cairns et al., 1996; Siddall et al., 2003).

### *Pain Models After SCI*

In animal models, pain soon after a spinal cord injury has been shown to decrease plasticity, reduce recovery, and increase indices of pain (Grau et al., 2017). Two common methods for modeling nociceptive input (pain) after SCI include intermittent electrical stimulation to the leg or tail at an intensity that activates c-fibers and capsaicin injected into the plantar paw (Grau et al., 2017). The benefit of using electrical stimulation is that researchers can engage nociceptive fibers without inducing tissue damage. Electrical stimulation also allows for good temporal control over the afferent signal. Additionally, there is a large body of work using this form of stimulation that have shown both how fiber type is related to shock intensity and how temporal variables, such as shock continuity and regularity, impact recovery (Grau et al., 2017). The benefit of capsaicin is that it provides more ecologically valid method of studying pain because pain is often longer lasting and is accompanied by tissue damage and inflammation (Caterina et al., 1997).

### *Pain After in a Transection Model of SCI*

Using a transection model of SCI, researchers have shown that nociceptive stimulation below the level of injury is detrimental to spinal cord plasticity (Grau, Barstow, & Joynes, 1998). In these studies, plasticity was examined using an instrumental learning paradigm described in detailed in Grau et al. (1998). In brief, transected animals were placed in tubes with a contact electrode secured to the plantar surface of the paw. A stimulating electrode was placed in the tibialis anterior muscle, which when stimulated, produced a flexion force of 0.4 N. A salt solution was then

placed below the animal's limb. After three shocks, the water level was adjusted so that the contact electrode was 4 mm under the surface. Animals would then be trained with either contingent stimulation (Masters) or noncontingent stimulation (Yoked). In Master animals, shock could be avoided by maintaining the leg in a flexed position. The Yoked animals acted as a control for the number and pattern of shock, but the shock was not contingent on their leg position. Instead these animals received shock in the same pattern as the Master rats, but regardless of their leg position. Results show that only Master subjects, which have control over the shock, learn to maintain their leg in a flexed position (Grau et al., 1998). Conversely, the Yoked subjects failed to learn to maintain their leg in a flexed position. Additionally, when Yoked controls are later exposed to Master stimulation, they continue to fail despite having control of shock (Grau et al., 1998). This is reminiscence of learned helplessness (Seligman & Maier, 1967). These studies suggest that noxious uncontrollable stimulation is detrimental after SCI.

In order to explore the adverse properties of uncontrollable stimulation, researchers derived a shock schedule that simulated the pattern of stimulation received by Yoked animals (Baumbauer et al., 2008). This produced a variable stimulation pattern with a mean interstimulus interval (ISI) of two seconds. When subjects were exposed to this intermittent stimulation at an intensity that engaged a robust C-fiber response, subjects showed a similar inability to learn when exposed to Master stimulation (Grau et al., 1998). This effect lasted for up to 48 hours (Crown, Ferguson, Joynes, & Grau, 2002). A further examination of the parameters found that intermittent stimulation given at an intensity of 1.5 mA and for a duration of at least six minutes induced a form of

maladaptive plasticity that enhanced nociceptive reactivity and impaired spinal cord learning (Baumbauer et al., 2008).

### *Pain in a Contusion Model of SCI*

Using a rat contusion model, six minutes of nociceptive stimulation given the day after a SCI increased tissue loss at the site of injury (Grau et al., 2004; Turtle et al., 2017). Additionally, animals showed decreased locomotor scores, decreased weight gain and increased mortality across the 42-day recovery period (Grau et al., 2004; Turtle et al., 2017). In another study, nociceptive stimulation given the day after injury was found to increase indices of pain beginning 24 hours after treatment and lasting out to 28 days. Analysis of the underlying cellular mechanisms found that nociceptive stimulation decreased the expression of BDNF and its receptor TrkB, activated cell death pathways (caspase 1, 3, 8), increased the expression of pro-inflammatory cytokines (NFκB, TNFα, IL-1β), and increased hemorrhage within 24 hours after stimulation (Garraway et al., 2011; Garraway et al., 2014; Grau et al., 2017; Turtle et al., 2017). The vulnerability of the spinal cord to the detrimental effects of noxious input waned after the first week after injury (Grau et al., 2004). Interestingly, this matches the time course of blood spinal cord barrier (BSCB) disruption seen after SCI (see hemorrhage section for more details). A similar pattern of results is seen with capsaicin treatment after SCI (Turtle et al., 2015).

Researchers have also examined instrumental learning in contused animals in order to determine whether noxious stimulation given in a controllable manner would be detrimental after a contusion injury (Grau et al., 2004). This is an important question, as stimulation is used after SCI in humans during rehabilitation, often with mixed results

(Ragnarsson, 2008). Studies examining the impact of Master and Yoked stimulation found, similar to transected subjects, that only Master subjects learned to maintain a flexion response (Grau et al., 2004). When contused animals were trained with Yoked stimulation, Yoke subjects showed a deficit in locomotor recovery that was evident six weeks after injury (Grau et al., 2004). Additionally, Yoked subjects showed increased spasticity and lesion volume. This study suggests that noxious stimulation that is uncontrollable is detrimental after injury.

A few studies have examined pharmacological treatments aimed at preventing the detrimental effects of pain input after SCI (Hook et al., 2007; Turtle et al., 2017). The first study examined whether morphine could prevent these effects (Hook et al., 2007; Turtle et al., 2015). While morphine produced sufficient analgesia to block pain input to the brain, it did not block the detrimental effects of nociceptive input on locomotor recovery. Additionally, morphine administration increased morbidity and increased pain reactivity in subjects receiving nociceptive input (Hook et al., 2007). In another study, a local injection of lidocaine was used to block nociceptive input (Turtle et al., 2017). In this study, lidocaine blocked the decrease in locomotor behavior and the increase in lesion volume seen after nociceptive stimulation. Additionally, within three hours after lidocaine treatment, shock-induced indices of hemorrhage, inflammation and cell death were all attenuated (Turtle et al., 2017). This suggests that blocking nociception induced excitation is sufficient to block the deficit in recovery.

## Progressive Hemorrhagic Necrosis

After a SCI, a hemorrhage often develops due in part to the shearing of blood vessels during the initial traumatic event (Tator, 1995). However, in a subset of SCI patients there is an expansion of this hemorrhage (Tator, 1995). This condition is known as progressive hemorrhagic necrosis (PHN) (Simard et al., 2007). PHN is thought to be the consequence of capillary breakdown due to a lack of structural integrity. This breakdown leads to an increase in red blood cell infiltration and capillary fragmentation (Simard et al., 2007). The infiltration of red blood cells into spinal tissue causes mass cell death due to their toxicity with neural tissue. A key mechanism in the development of PHN is the formation of the SUR1-TRPM4 complex (Simard et al., 2007). This complex is formed in response to depleted intracellular ATP. Once formed, this complex leads to cell depolarization, cytotoxic edema, and cell death (Simard et al., 2007). Blocking the formation of this channel after SCI prevents the capillary fragmentation and red blood cell infiltration, leading to better locomotor recovery and tissue sparing (Simard et al., 2007).

In a recent study, nociceptive stimulation was found to induce PHN after SCI (Brumley, Turtle, Forsberg, & Grau, 2016). Using both spectrophotometry and immunohistochemistry, this study found that six minutes of electrical stimulation or an injection of capsaicin in the paw caused an increase in hemorrhage within an hour after treatment. The peak hemorrhage was seen at three hours for shock and at 24 hours for capsaicin. Additionally, electrical stimulation was found to increase the formation of the SUR1-TRPM4 complex and infiltration of red blood cells in the lesion cite.

Hemorrhage expansion may also be initiated by changes in blood pressure after SCI (Guha, Tator, & Rochon, 1989). In one study, adrenaline was used to increase the blood pressure beginning 30 minutes after SCI (Guha et al., 1989). It was found that hypertension (180 mmHg), increased hyperemia. Given the lack of spinal cord blood flow, it was concluded that this likely caused an increase in hemorrhage and edema. Similarly after TBI and stroke hypertension has been shown to increase hemorrhage and blood brain barrier permeability (Hardebo & Beley, 1984; Ito et al., 1980).

## Blood Pressure and SCI

Blood pressure around the time of injury is one of the strongest predictors of locomotor recovery after thoracic SCI in rats (Nielson et al., 2015). A retrospective study in humans similarly showed that pre-existing hypertension was correlated with lower motor scores (Kepler, Schroeder, et al., 2015). This is of particular concern because the increase in average age at the time of SCI is associated with increased hypertension (DeVivo & Chen, 2011; Furlan et al., 2005).

After SCI, blood pressure regulation can be disrupted due in part to the loss of or damage to descending sympathetic fibers (Rabchevsky et al., 2012). This can cause a shift in the balance between the sympathetic and parasympathetic nervous systems. Initially after injury, there is a brief hypertensive spike along with a decreased peripheral resistance (yielding an increase in blood flow) that is associated with a surge of norepinephrine (Gondim et al., 2004). This is then followed by marked hypotension and bradycardia as sympathetic activity decreases (Phillips et al., 1998). The loss of autonomic control is especially dangerous in people with a SCI above thoracic nerve 6

(T6) who are prone to develop a condition known as autonomic dysreflexia (Eldahan & Rabchevsky, 2017). During an episode of autonomic dysreflexia, SCI patients experience a sudden and severe increase in blood pressure that can be deadly if left untreated (Eldahan & Rabchevsky, 2017).

While a number of drugs have been used for treating autonomic dysreflexia, few controlled trails have been conducted (Krassioukov, Warburton, Teasell, Eng, & Spinal Cord Injury Rehabilitation Evidence Research, 2009). The one drug currently approved for the management of autonomic dysreflexia is prazosin (Krum, Louis, Brown, & Howes, 1992). Prazosin is selective blocker of the alpha-1 adrenoreceptor, which acts to relax blood vessels (Krum et al., 1992), and has been shown to reduce the occurrence and severity of autonomic dysreflexia episodes. In another study examining the rise of blood pressure during sexual stimulation, prazosin treated participants showed significant lower rises in systolic blood pressure during stimulation (Phillips, Elliott, Zheng, & Krassioukov, 2015). Prazosin has also been shown to help with the relaxation of the bladder and decrease motoneuron excitation by modulating calcium-mediated currents (Kontani & Hayashi, 1997; Rank et al., 2011).

## Specific Aims

In the current study, I explored the relationship between noxious input and hemorrhage. Further, I evaluated whether changes in blood pressure could account for the increase in hemorrhage seen after noxious input in both sexes. The central hypothesis is that noxious input causes a change in blood pressure, leading to a breakdown of the capillaries and an expansion of the secondary hemorrhage.

Aim 1 (Chapter III) determined whether stimulation given soon after injury or briefer and/or less intense periods of stimulation have an acute effect on hemorrhage. Aim 2 (Chapter IV) examined whether blood pressure changes after electrical stimulation were correlated with hemorrhage and locomotor performance. Aim 3 (Chapter V) examined blood pressure after a capsaicin injection. Aim 4 (Chapter VI) determined if blood pressure changes were seen after noxious controllable stimulation. Aim 5 (Chapter VII) evaluated whether males and females showed an increase in hemorrhage and blood pressure after nociceptive activation. Finally, Aim 6 (Chapter VIII) examined whether changes in blood pressure caused hemorrhage expansion and the detriment in locomotor performance.

# CHAPTER II

# GENERAL METHOD

## Subjects

Adult Sprague Dawley (200-400 g) rats were obtained from Envigo (Houston, TX). Upon arrival to the vivarium, animals were dual housed in a room with a 12-hour light-dark cycle with food and water *ad libitum*. Animals were acclimated to the room for at least seven days before experimentation. Experiments were carried out during the light cycle in accordance with NIH standards for the care and use of laboratory animals, and were approved by the University Laboratory Animal Care Committee at Texas A&M University (Council & Research, 2011). Every effort was made to minimize suffering and limit the number of animals used.

## Contusion Surgery

Using the New York University (NYU) Multicenter Animal Spinal Cord Injury Study (MASCIS) device, rats received a contusion at the T12 vertebral level. For the surgery, animals were anesthetized with a mixture of 5% isoflorane and medical oxygen. Concentrations of isoflorane were maintained at 2-3% isoflorane during surgery. An initial incision was made through the skin centered over the T12 vertebra. Next, two longitudinal incisions (six centimeters in length) centered over the T12 vertebra were made on both sides of the spinal column. The T12 vertebra was then exposed and a laminectomy was performed. The spinal column was secured into the MASCIS device and the impactor centered on the lesion site. A 10-gram weight was then dropped from a

height of 12.5 mm with a five second dwell time. Rats receiving a sham operation went through all of the steps of the surgery except the weight was not dropped. After surgery, Michel clips were used to close the skin incision. Animals then received an injection of 100,000 units/kg of penicillin and three milliliters of saline to prevent infections and compensate for fluid loss. Subjects were then placed in a temperature-controlled recovery room (25°C) and allowed to recover overnight (24 hours) with food and water *ad libitum*. Bladders were voided twice daily and prior to any experimental procedures. After experimentation was finished, animals were euthanized with a lethal dose of pentobarbital (100mg/kg; i.p.).

**Locomotor Performance**

Before surgery, rats were acclimated on three separate days to a 45-inch plastic pool (Target) for four minutes. Beginning the day after contusion, rats were analyzed using the Basso, Beattie, and Bresnahan (BBB) locomotor scale. To obtain the BBB score, the subject was placed in an observation arena (a 48 cm wide children swimming pool) and locomotor performance was assessed for four minutes by a trained observer that was unaware of the animal's treatment condition. BBB scores were resamples at later time points during each experiment to examine changes in locomotor performance. For long-term recovery studies, BBB scores were analyzed for the first seven days, at ten days, and then once a week until day 21.

**Blood Pressure**

Before surgery, rats were acclimated on three separate days to the tubes used to restrain subject during the assessment of blood pressure and testing procedures. Testing

was done in a warm room (27°C) with dim lighting. Subjects were placed in a clear acrylic tube with a black adjustable nose cone atop a Far-infrared warming platform (Kent Scientific). Tail temperature was monitored with an infrared thermometer to insure temperatures reached and were maintained between 32-35°C. A warming blanket was placed over the tail, when necessary, to increase the temperature. If animals got warm or started to wiggle, a dampened paper towel was placed over the tube to lower body temperatures. After acclimating in the tubes untouched for five-minute, the occlusion cuff and volume pressure recording (VPR) sensor was carefully glided up the tail and secured at the base of the tail to the tube. If any resistance was meet when sliding the cuffs to the base of the tail, a larger cuff or sensor was chosen. Animals were then acclimated to the cuffs for another five minutes before blood pressure measurements were obtained. Blood pressure measurements were obtained using the CODA High Throughput Noninvasive Blood Pressure system and data acquisition software. The program was set to automatically inflate and deflate the cuffs for 15 consecutive cycles with 5 seconds between cycles. The maximum occlusion pressure was set at 250 mmHg with a deflation time of 20 seconds and a minimum volume of 15 μL. Acquisition of the blood pressure lasted approximately 10 minutes. During blood pressure acquisition, tail and body temperature were regularly monitored. If for any reason the animal did not have at least four accepted blood pressure readings and one accepted heart rate reading, the animal would receive an additional five trials immediately following the last regular trial. If animals began to show signs of agitation, placing a dampened paper towel over their tube often calmed them down. If this did not

work, the testing was paused and the animal was removed from the tube. After the assessment of blood pressure and heart rate, the occlusion cuff and VPR sensor were taken off the tail and subjects were removed from the restraint tubes. Finally, animals had their bladders voided before being placed back in their home cage.

### Drug Preparation and Administration

Prazosin (3 mg/kg) was dissolved in 30% glucose. Due to the low solubility of prazosin, hydrochloric acid was added when dissolving the drug. Once dissolved, the solution was adjusted to pH 7 and brought up to the final volume. Prazosin was delivered by an intraperitoneal (i.p.) injection with a volume of 2 mL/350 gram.

Norepinephrine (1 mg/kg) was dissolved in 0.9% saline and adjusted to pH 7. Norepinephrine was injected subcutaneously (s.c.) with a volume of 2 mL into the rat's lower flank.

Capsaicin (3%) was dissolved in a solution of 7% Tween-80 and 23% saline (0.9% NaCl). Due to the low solubility of capsaicin, it had to be slightly heated and vortexed immediately before administration. Capsaicin was administered using an intradermal (i.d.) injection into the dorsal surface of the paw at a volume of 0.05 mL.

### Uncontrollable Tail Shock

Rats were setup for uncontrollable tail shock as previously described in Crown et al. (2002). In brief, animals were restrained in opaque Plexiglas tubes with their hindlimbs hanging freely in a sound proof box. An electrode was placed an inch and a half from the tip of the tail and secured with porous tape. Electrode gel was used to ensure the tail maintained a good contact with the electrode surface. Electrical

stimulation was given in a variable spaced pattern (80 ms pulse, ISI 0.2-3.8 seconds, 60 Hz AC current). Typically, 180 stimulations were given at an intensity of 1.5 mA, although this varied across studies. Unshocked controls were restrained in tubes with the electrode attached for an equivalent duration, but no shock was administered.

**Instrumental Learning**

Rats were loosely restrained in opaque Plexiglas tubes, which allowed the legs to hang freely. The leg was then set up for instrumental learning as described in Grau et al. (2004). A thin wire electrode was placed into the tibialis anterior muscle 3.2 cm above the ankle. The other lead consisted of a stainless steel wire looped through the skin 1.5 cm above the ankle (1.7 cm below the other lead). An eight-centimeter contact electrode was secured with porous tape to the plantar side of the paw. The leg was then taped forward to inhibit extraneous movements. Once the leg was set up, a small glass dish filled with a soupy salt solution was placed under the subject's leg. The animal then received three quick shocks (0.6 mA) to the tibialis anterior muscle to insure proper placement. The water level was then set so that the contact electrode resided 4 mm under the water. The leg used for testing was counterbalanced across groups. To monitor leg position, an electrode was placed in the water. Whenever the contact electrode touched the salt solution, it completed a circuit that was measured by the computer.

Instrumental training lasted a total of 30 minutes. Master and Yoked rats were always run in pairs. For subjects receiving Master shock, electrical stimulation to the tibialis anterior muscle was given whenever the foot touched the underlying salt solution. Because contused animals learn very quickly, salt solution was added to the

underlying dish when the Master subject learned to maintain their leg above the solution for two consecutive minutes within the first ten minutes of training. Solution was added in increments of 25 mL (i.e., 2 mm increase) until the contact electrode touched the underlying solution. For subjects receiving noncontingent shock (Yoked), animals received shock whenever the Master subject's foot touched the underlying solution and solution level was adjusted at the same time and by the same amount. As a control, a group of animals remained unshocked. Unshocked rats had their leg set up as outlined above, however after receiving the three shocks used to set the water level, the animal received no other shocks and remained in the tubes for thirty minutes.

A the end of training, the computer compiled data into one-minute time bins. Learning was defined as an increase in flexion duration. Flexion duration was calculated for each time bin using the following formula: (60 seconds minus time in solution$_i$ ) / (Response Number$_i$ + 1), where i is the current time bin.

**Intradermal Capsaicin Injection**

Subjects were restrained in opaque Plexiglas tubes with their hindlimbs hanging freely. Capsaicin (3%) was then injected intradermally (i.d.) into the dorsal paw using a 27-gauge needle. Controls received an i.d. injection of vehicle (7% Tween-80 in 23% saline). To maintain consistency across treatment conditions and with past studies, capsaicin and vehicle treated animals remained in the tubes for six minutes after injection.

## Tissue Collection for Protein Analysis

After a lethal dose of pentobarbital, a one-centimeter section of spinal cord tissue was extracted and flash frozen in liquid nitrogen. Samples were stored at -80°C until processed. Tissue was processed for protein isolation using QIAzol lysis reagent according to the manufacturer's instructions.

## Spectrophotometry

A 1.5 μL sample of the protein extract (before it was diluted with Laemeli buffer) was loaded into a spectrophotometer (Thermo) and a full spectrum analysis was run. Absorbance was analyzed at 420 nm for hemoglobin.

## Immunoblotting

A Bradford assay was used to quantify protein concentration of each sample. Samples were then diluted with 4X Laemeli buffer to a final concentration of 3 μg/μL, except for Experiment 1 where the concentration was 2 μg/μL.

Western blots were run on pre-cast 26-well Criterion gels (BIORAD). Briefly, protein samples in 4X Laemeli buffer were heated to 96°C for 10 minutes before 10 μL of each sample was loaded into the wells. Electrophoresis was then performed at 180V for approximately an hour and fifteen minutes. Proteins were then transferred to a PVDF membrane for one hour at 100V. Membranes were then placed in milk for one hour before being placed overnight in primary antibody (hemoglobin-α, 1:1000) at 4°C. The next day, membranes were washed several times with TBST before incubation for one hour in secondary antibodies (goat anti-rabbit, 1:5000) at room temperature. Finally, blots were imaged using Enhanced Chemiluminescence (ECL).

## Statistics

SPSS was used to perform all statistical tests. Significance was set at 0.05. *Post hoc* comparisons were conducted using Fisher's Least Statistical Difference (LSD) test. For missing values, an average of the adjacent cells was used to enable statistical analysis.

# CHAPTER III

# SHOCK PARAMETERS THAT INDUCE THE EXPANSION OF THE HEMORRHAGE

Work examining the effect of nociceptive stimulation in a contusion model of spinal cord injury showed that six minutes of electrical stimulation to the tail at 1.5 milliamps (mA) produced a long-term impairment in locomotor recovery and an expansion of the lesion volume (Grau et al., 2004). Research also showed that the effect of shock waned after the first week (Grau et al., 2004). This matches the disruption in the blood-spinal cord-barrier (BSCB) seen within an hour after a spinal cord injury, which was restored by 14 days after injury (Figley, Khosravi, Legasto, Tseng, & Fehlings, 2014). An increase in the indices of hemorrhage has also been observed after noxious input in spinally contused animals (Turtle et al., 2017).

The current set of experiments explored the stimulus conditions that induce hemorrhage and the acute effect on locomotor performance. Experiment 1 examined whether the time since injury impacted the expansion of the secondary hemorrhage. Experiment 2 explored whether a shorter duration of stimulation caused an expansion of the hemorrhage and whether this correlates with changes in locomotor performance. Finally, Experiment 3 examined whether less intense shock caused a change in hemorrhage and locomotion.

## Experiment 1: Effect of Shock Exposure on Different Days Post Surgery

In this study, I examined the effect of electrical stimulation given on day one, four, or 14 after spinal cord injury on hemorrhage expansion. These intervals were selected based on previous work exploring how the effect of nociceptive stimulation varies over time after injury (Grau et al., 2004).

### *Procedure*

Male Sprague Dawley rats (n = 6) received a moderate spinal cord contusion at T12. The next day, baseline BBB scores were obtained. Subjects were then randomly assigned to receive shock or remain unshocked on day one, four or 14 post-injury. Groups were balanced using BBB scores obtained 24 hours after injury. On the designated day of treatment (post-injury day one, four, or 14), subjects received either six minutes of uncontrollable electrical stimulation (1.5mA, 0.2-3.8 second ISI) to the tail or remained unshocked. Locomotor performance was then monitored daily using the BBB locomotor scale. Additionally, locomotor scores were assessed three hours after stimulation to assess whether shock had an immediate effect on locomotor performance. After obtaining the three-hour BBB score, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm) and examined by gel electrophoresis and immunoblotting for hemoglobin.

*Results*

BBB score did not differ between groups prior to treatment. BBB scores obtained the day after surgery, prior to treatment, ranged from 4.67 (± 0.49) to 5.25 (± 0.59). An ANOVA found no differences between groups, $F < 1.0$, $p > 0.05$.

BBB locomotor scores did not change in response to stimulation. BBB scores were taken daily and at three hours post-stimulation. After controlling for baseline variability using an ANCOVA, BBB scores obtained prior to treatment on the day of testing (day one, four or 14) were significantly different across groups ($\overline{x} = 1.75$, 2.6, and 6.8, respectively), all $F$s > 8.73, $p < 0.05$ (Figure 1). To control for these differences, a change score was calculated by subtracting the BBB score obtained three hours after shock from the BBB score obtained prior to treatment on the day of testing (day one, four or 14). An ANCOVA with day of treatment and shock as the between subjects variables, the change score as the within subjects variable, and baseline scores as a covariate, revealed no significant differences, all $F$s $< 1.72$, $p > 0.05$ (Figure 1).

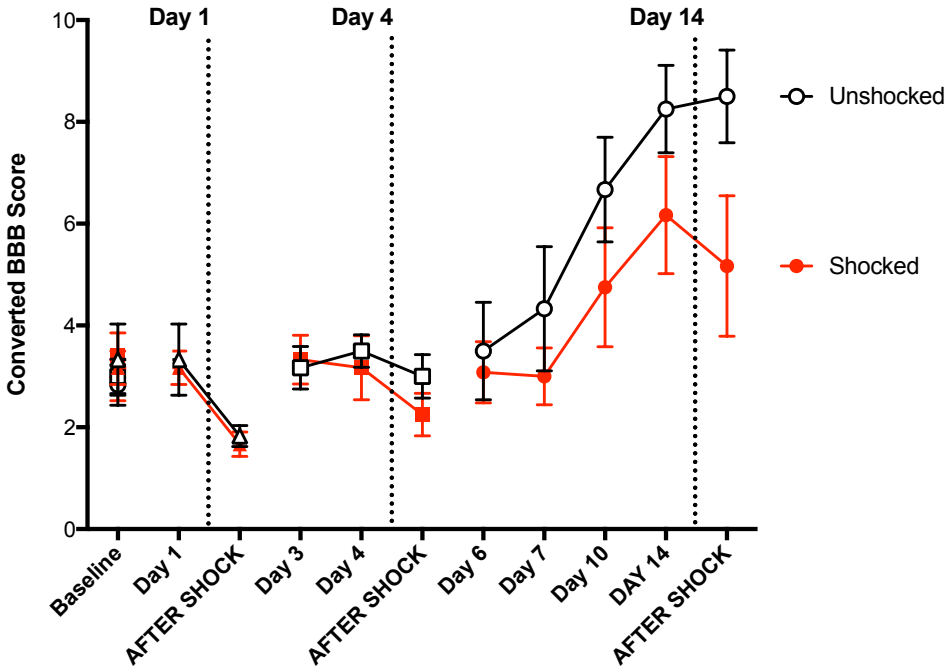


**Figure 1. Locomotor performance three hours after electrical stimulation given on different days.** BBB locomotor scores taken at three hour after stimulation did not differ across groups. Error bars represent SEM (n = 6).

Stimulation soon after injury increased indices of hemorrhage three hours after stimulation. The peak absorbance for hemoglobin (420 nm) was analyzed using an ANOVA with day of treatment and shock as the between subjects variables. The overall analysis revealed a significant main effect of day of treatment and shock, all $F$s > 4.31, $p$ < 0.05 (Figure 2A). No other effects were statistically significant, $F$ < 1.09, $p$ > 0.05. *Post hoc* comparisons of the main effects revealed an increase in hemorrhage in subjects that received shock. Additionally, hemorrhage was significantly greater on day one compared to day four and 14. There was also a significant increase in hemorrhage on day four compared to day 14.

The levels of hemoglobin-α within the spinal cord tissue were not significantly different between groups. Using quantitative Western blotting, the level of hemoglobin protein at 15 kDa was analyzed with an ANOVA with day of treatment and shock as the between subjects variables. The overall analysis found no statistical differences, $F$ < 1.0, $p$ > 0.05 (Figure 2B).

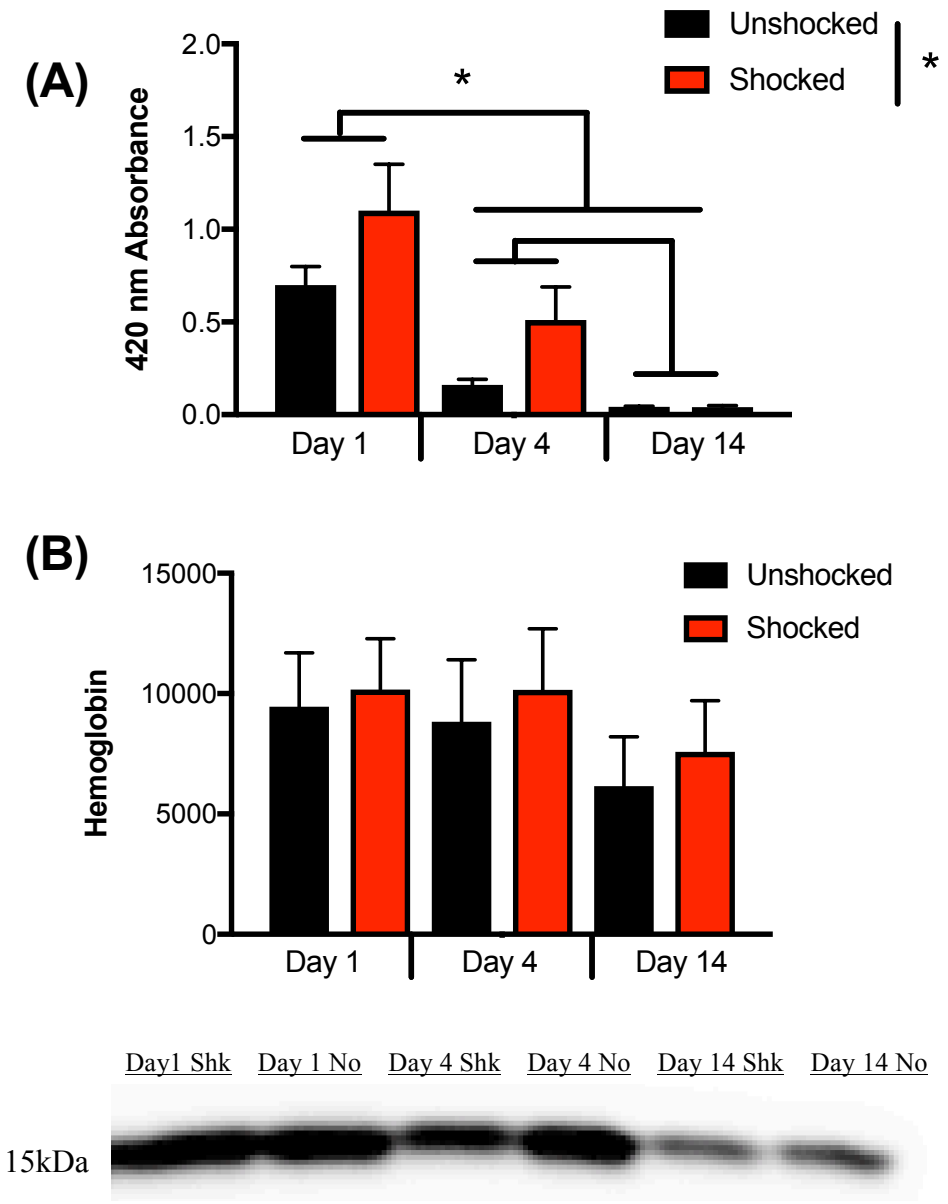


**Figure 2. Hemorrhage three hours after electrical stimulation given on different days.** (A) The absorbance at 420 nm in spinal cord tissue collect at three hours after stimulation was higher in shocked subjects. Further, hemorrhage decrease as the day post-surgery increased. (B) The amount of hemoglobin-α did not differ between groups. Error bars represent SEM (n = 6).

*Discussion*

The present experiment is in agreement with prior work showing that nociceptive stimulation produces a larger impairment when given soon after injury (Grau et al., 2004). Here I found a significant change in the magnitude of hemorrhage, as measured by peak absorbance at 420 nm. This replicates and expands the results of our previous study showing that nociceptive stimulation given 24 hours after injury increases indices of hemorrhage and that these effects waned when shock was given later after injury (i.e., by day 14). Thus, it is likely that the impaired recovery and expansion of the lesion seen in the previously published study was due in part to an increase in hemorrhage. Treatments targeting this increase in hemorrhage need to focus on the acute phase of injury as our results indicated that the spinal cord was most vulnerable soon after injury.

## Experiment 2: Effect of Shock Duration on Incidence of Hemorrhage at Three Hours

In this experiment, I examined whether briefer periods of stimulation (0, 14.5, 72, or 360 seconds; 1.5 mA) could produce an expansion of the hemorrhage and a deficit in locomotor behavior within the three hours post-stimulation period. Because six minutes (360 seconds) has been shown to produce a lasting deficit when given at 1.5 mA, it was used here as a positive control (Grau et al., 2004). Additionally, in this experiment, I examined locomotor performance at multiple time points after stimulation.

### *Procedure*

Twenty-four hours after receiving a moderate spinal cord contusion at T12, male Sprague Dawley rats (n = 8) had their locomotor scores evaluated using the BBB locomotor scale. Subjects were then randomly assigned to receive uncontrollable electrical stimulation (1.5mA, 0.2-3.8 second ISI) with durations of 0, 14.5, 72, or 360 seconds. Groups were balanced using BBB scores obtained 24 hours after injury. To increase sensitivity and better detect changes in locomotor behavior, BBB scores were examined at one, two, and three hours after stimulation. After obtaining the three-hour BBB score, subjects were sacrificed and a one-centimeter section of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm) and examined by gel electrophoresis and immunoblotting for hemoglobin.

*Results*

BBB score did not differ between groups prior to treatment. BBB scores obtained the day after surgery, prior to treatment, ranged from 4.38 (± 0.52) to 4.75 (± 0.43). An ANOVA revealed no differences between groups, $F$ (3, 28) < 1.0, $p$ > 0.05.

Locomotor performance declined as shock duration increased. BBB scores were obtained at one, two, and three hours after stimulation to examine whether the length of shock exposure affected locomotor scores. A repeated measures ANCOVA was used with duration as the between subjects variable, baseline BBB scores as the covariate, and time as the repeated measure. Analysis revealed a main effect of duration, $F$ (3, 27) = 6.33, $p$ < 0.01 (Figure 3). No other groups were statistically significant, all $F$s < 1.0, $p$ > 0.05. *Post hoc* analysis of the main effect showed that longer durations of stimulation (72 and 360 seconds) significantly impaired locomotor performance compared to subjects given shorter stimulation durations (0 and 14.5 seconds).

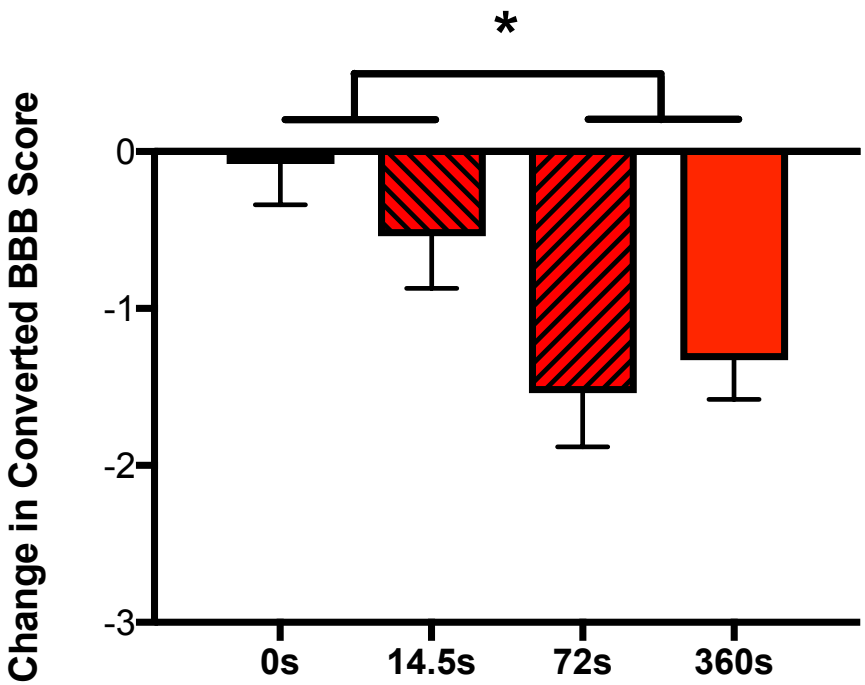


**Figure 3. Locomotor performance over the three hours after different durations of electrical stimulation.** BBB locomotor scores over the three hours post stimulation period significantly decreased with stimulation durations 72 seconds and longer. Error bars represent SEM (n = 8).

Exposure to longer durations of stimulation increased indices of hemorrhage three hours after stimulation. The absorbance peak for hemoglobin (420 nm) was analyzed using an ANOVA with duration as the between subjects variable. Overall analysis revealed a main effect of duration, $F$ (3, 28) = 2.96, $p < 0.05$ (Figure 4A). Post-hoc analysis of the main effect indicated a significant increase in hemorrhage for subject receiving 72 and 360 seconds of stimulation compared to subject receiving 0 seconds of stimulation. No other groups were statistically significant.

The level of hemoglobin-α within the spinal cord tissue did not change with shock duration. Using quantitative Western blotting, the level of hemoglobin protein at 15 kDa was analyzed with an ANOVA with duration as the between subjects variable. There were no significant effects, $F < 1.0$, $p > 0.05$ (Figure 4B).

*Discussion*

This study replicated the prior work showing that 360 seconds of nociceptive input increases indices of hemorrhage (Turtle et al., 2017). Additionally, this experiment revealed that shock durations as short as 72 seconds can inhibit locomotor behavior and potentiate the expansion of the hemorrhage. This suggests that even brief periods of nociceptive stimulation after a spinal cord injury may be detrimental. Supporting this, an unpublished study in our lab has shown that 72 seconds of stimulation shortly after injury can inhibit locomotor recovery out to 42 days. Given these results, researchers and clinicians should aim to minimize the duration of nociceptive input people with SCI receive.

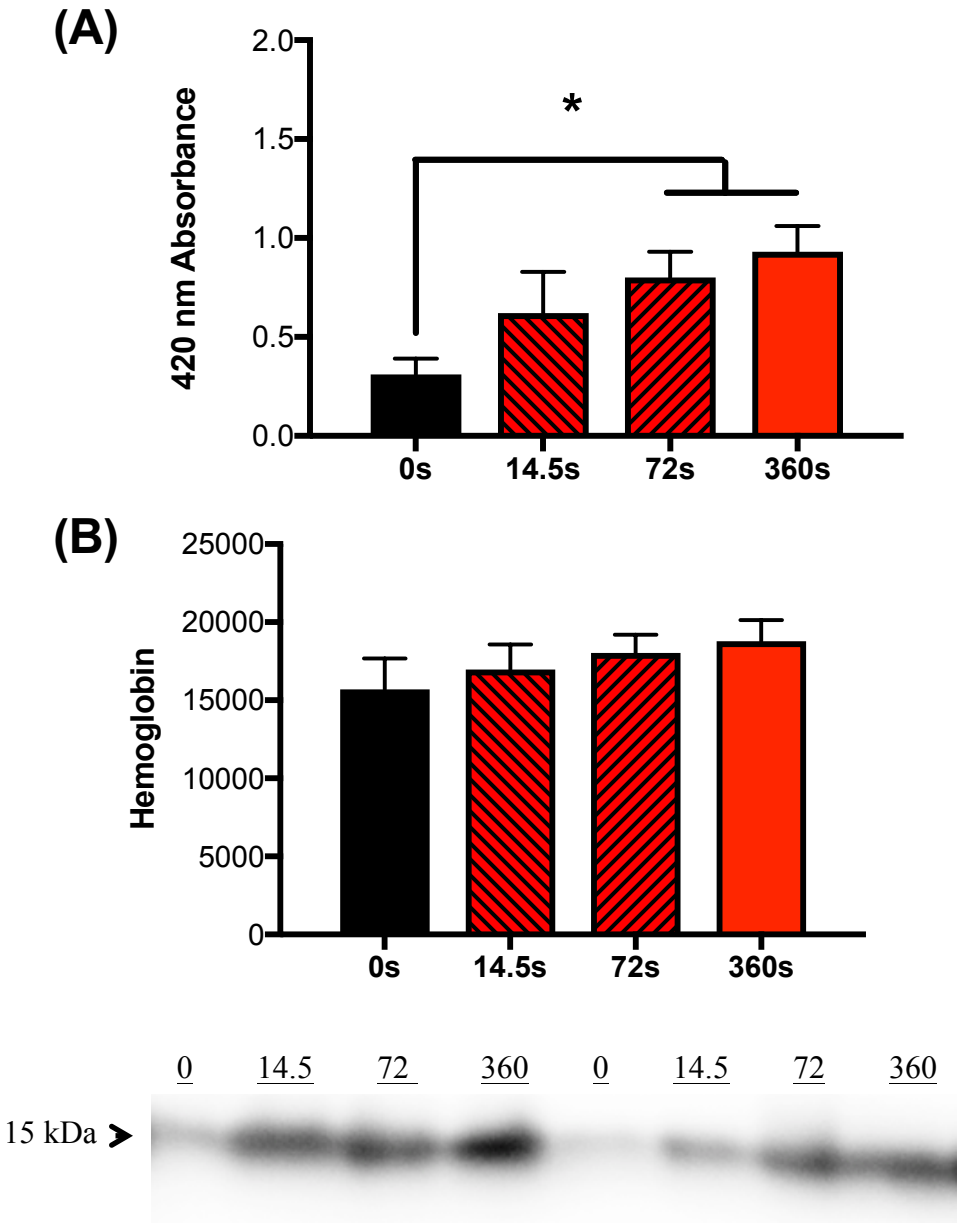


**Figure 4. Hemorrhage three hours after different durations of electrical stimulation.** (A) The absorbance at 420 nm taken at three hours post stimulation increased as the duration of stimulation increased. (B) The amount of hemoglobin-α did not differ between groups. Error bars represent SEM (n = 8).

## Experiment 3: Effect of Shock Intensity on Incidence of Hemorrhage at Three Hours

In this experiment I examined whether six minutes of stimulation given at a lower intensity (0, 0.17, 0.5, or 1.5 mA) would produce a similar expansion of the hemorrhage and change in locomotor behavior within the three hours post-stimulation period. For a positive control, 1.5 mA was used.

### *Procedure*

Male Sprague Dawley rats (n = 8) received a moderate spinal cord injury at T12. The next day, baseline BBB scores were obtained. Subjects were then randomly assigned to receive six minutes of uncontrollable electrical stimulation (0.2-3.8 second ISI) to the tail at four different intensities: 0, 0.17, 0.5, or 1.5 mA. Baseline BBB scores, obtained 24 hours after injury, were used to balance groups. BBB scores were examined at one, two and three hours after stimulation to determine whether shock had an immediate locomotor effect. After obtaining the three-hour BBB score, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm) and examined by gel electrophoresis and immunoblotting for hemoglobin.

*Results*

BBB scores did not differ between groups prior to treatment. BBB scores obtained the day after surgery, prior to treatment, ranged from 5.06 (± 0.7) to 5.13 (± 0.67). An ANOVA found no difference between groups, $F(3, 28) < 1.0$, $p > 0.05$.

BBB scores were obtained at one, two, and three hours after stimulation to examine whether shock intensity affected locomotor scores. Higher stimulation intensities disrupted locomotor performance. A repeated measures ANCOVA with intensity as the between subjects variable, baseline BBB scores as the covariate, and time as the repeated measure found a main effect of intensity, $F(1, 27) = 42.01$, $p < 0.0001$ (Figure 5). No other effects were significant, all $F$s < 1.37 $p > 0.05$. *Post hoc* analysis of the main effect showed that higher stimulation intensities (0.5 and 1.5 mA) significantly impaired locomotor performance compared with subjects given lower intensities of stimulation (0 and 0.17 mA).

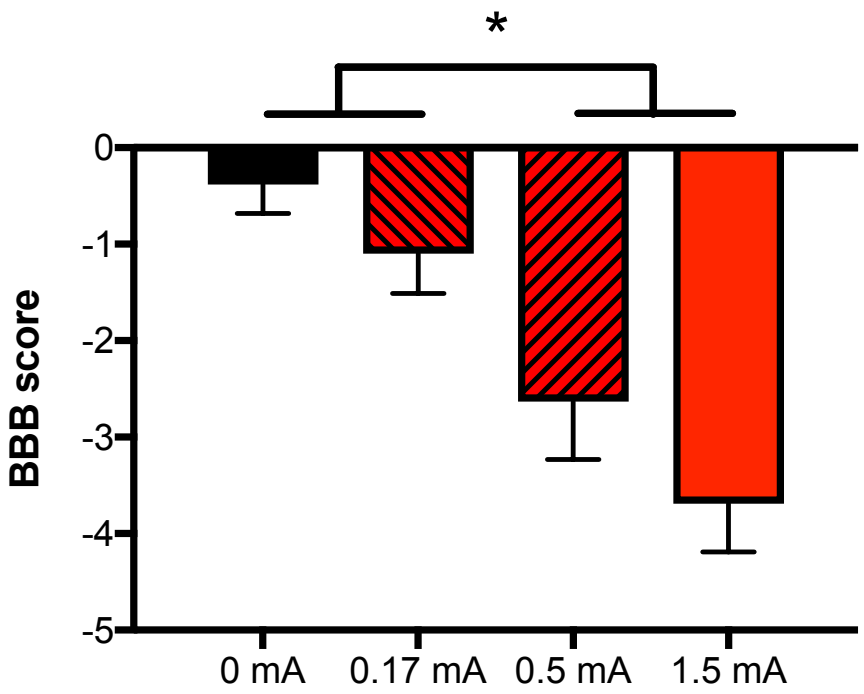


**Figure 5. Locomotor performance over the three hours after different intensities of electrical stimulation.** BBB locomotor scores taken during the three-hour post stimulation period decreased at intensities of 0.5 mA and above. Error bars represent SEM (n = 8).

Exposure to higher intensities of stimulation increased the indices of hemorrhage three hours after stimulation. The magnitude of the peak in absorbance at 420 nm was analyzed using an ANOVA with intensity as the between subjects variable. Omnibus analysis revealed a main effect of condition, $F$ (3, 28) = 5.16, $p < 0.01$ (Figure 6A). *Post hoc* analysis of the main effect found a significant increase in hemorrhage for subjects receiving 0.5 mA stimulation compared to subjects given low intensity stimulation (0 and 0.17 mA). There was a marginally significant difference between 0 and 1.5 mA, $p = 0.06$.

The level of hemoglobin-α within the spinal cord tissue was not changed with shock intensity. Using quantitative Western blotting, the level of hemoglobin protein at 15 kDa was analyzed with an ANOVA with intensity as the between subjects variable. There were no significant effects, $F < 1.0$, $p > 0.05$ (Figure 6B).

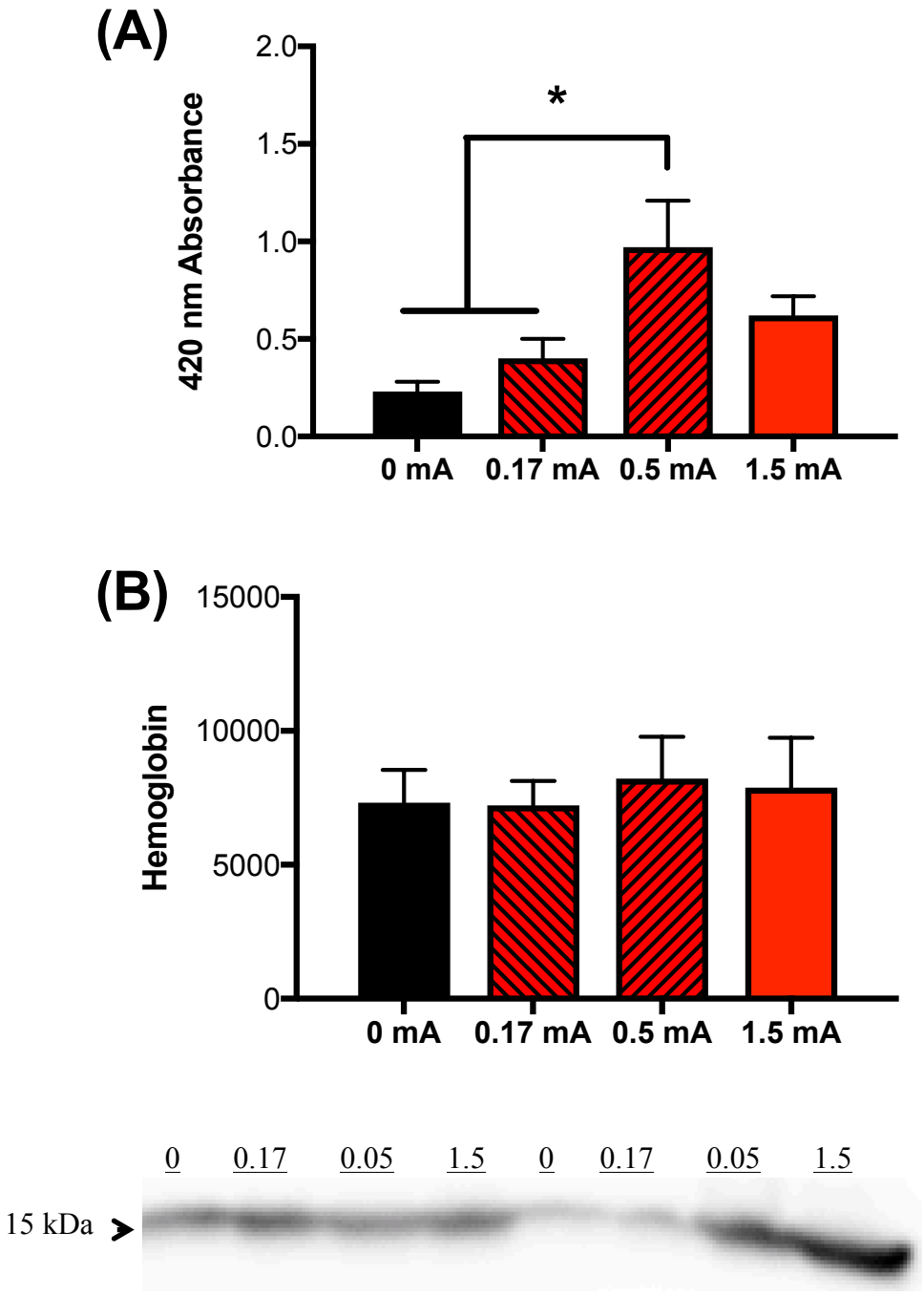


**Figure 6. Hemorrhage three hours after different intensities of electrical stimulation.** (A) The absorbance at 420 nm taken at three hours post stimulation increased as the intensity of stimulation increased. (B) The amount of hemoglobin-α did not differ between groups. Error bars represent SEM (n = 8).

*Discussion*

The current study replicated the previous work showing that stimulation given at 1.5 mA produces an increase in hemorrhage and a decrease in locomotor scores (Turtle et al., 2017). Further this experiment showed that lower stimulation intensities (0.5 mA) can also be damaging to performance and produce an increase in hemorrhage. This study was further supported by unpublished work from our lab showing that stimulation given at 0.5 mA can impair long-term locomotor recovery when given within 24 hours of injury. These studies highlight the importance of managing nociceptive input after SCI.

# CHAPTER IV

# THE EFFECT OF SHOCK ON BLOOD PRESSURE

The regulation of blood pressure is disrupted after a spinal cord injury, leading to an imbalance between the sympathetic and parasympathetic nervous system (Eldahan & Rabchevsky, 2017). This lack of blood pressure regulation in people with SCI can be a serious problem, leading to death if left untreated (Eldahan & Rabchevsky, 2017). Previous research has shown that mean arterial blood pressure around the time of injury was highly correlated with their later locomotor recovery (Nielson et al., 2015).

Recently, it was found that nociceptive stimulation increases the extent of the hemorrhage after spinal cord injury (Turtle et al., 2017) In other models of central nervous system injury, the expansion of the hemorrhage has been linked to hypertension that accelerates blood-brain barrier damage (Ito et al., 1980; Hardebo & Beley, 1984).

In the current set of experiments I examined whether the expansion of the hemorrhage produced by nociceptive stimulation after SCI was related to hypertension. Experiment 4 examined whether blood pressure and hemorrhage were increased within the first three hours after electrical stimulation. Experiment 5 examined whether blood pressure and hemorrhage were increased 24 hours after electrical stimulation. Both experiments also examined whether locomotor performance was correlated with changes in blood pressure and hemorrhage.

## Experiment 4: Effect of Shock on Blood Pressure at Three Hours

In this experiment, I examined whether shock produced a change in blood pressure, hemorrhage, and locomotor behavior.

### *Procedure*

Twenty-four hours after receiving a moderate spinal cord contusion at T12, male Sprague Dawley rats (n = 8) had their locomotor scores evaluated using the BBB locomotor scale and their baseline blood pressure measurements taken using a non-invasive blood pressure cuff. Subjects were then randomly assigned to receive six minutes of electrical stimulation (180 shocks, 1.5mA, 0.2-3.8 second ISI) to the tail or remained unshocked. Groups were balanced using baseline systolic blood pressure and BBB scores. BBB scores and blood pressure measurements were monitored immediately, one, two, and three hours after electrical stimulation. After obtaining the three hours BBB score and blood pressure measurement, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm) and examined by gel electrophoresis and immunoblotting for hemoglobin.

### *Results*

BBB scores did not differ between groups prior to treatment. BBB scores obtained the day after surgery, prior to treatment, ranged from 3.13 (± 0.26) to 3.38 (±

0.6). An ANOVA found no significant differences between groups, $F$ (1, 14) < 1.0, $p$ > 0.05.

Nociceptive stimulation given a day after injury caused a decrease in locomotor scores. BBB scores were obtained immediately, one, two, and three hours after stimulation to examine whether shock exposure affected locomotor scores. A repeated measures ANCOVA was used with shock as the between subjects variable, baseline BBB scores as the covariate, and time as the repeated measure. The overall analysis revealed a main effect of shock, $F$ (1, 13) = 11.94, $p$ < 0.01 (Figure 7). No other effects were statistically significant, all $F$s < 2.73, $p$ > 0.05. *Post hoc* analysis showed reduced locomotor scores in subjects receiving nociceptive stimulation.

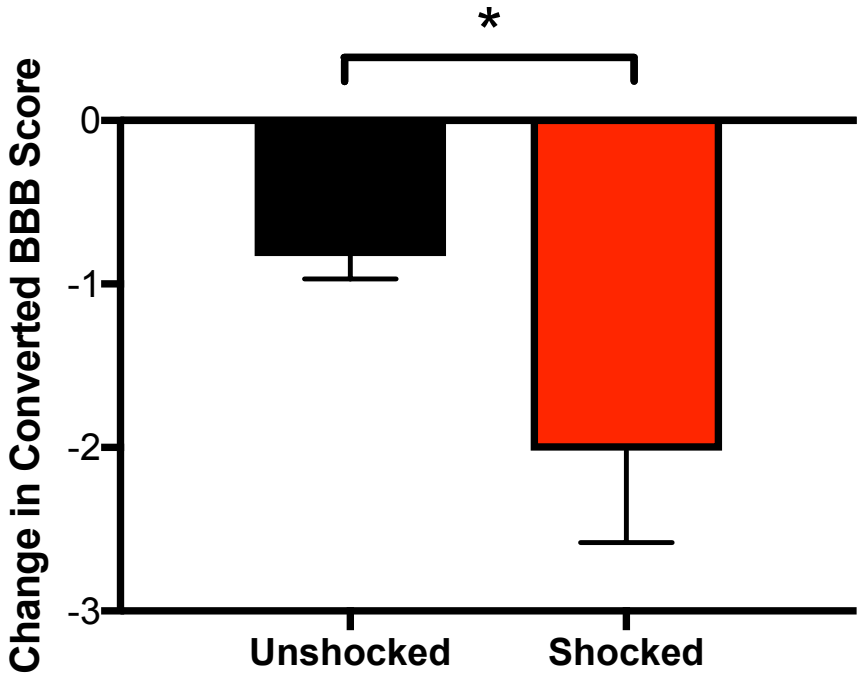


**Figure 7. Locomotor performance over the three hours after nociceptive stimulation.** BBB locomotor scores taken during the three hours post stimulation period decreased in subjects receiving stimulation. Error bars represent SEM (n = 8).

There was an increase in hemorrhage in subjects receiving nociceptive stimulation. The magnitude of the peak in absorbance at 420 nm was analyzed using an ANOVA with shock as the between subjects variable. Overall analysis revealed a main effect of shock, $F$ (1, 14) = 5.09, $p < 0.05$ (Figure 8). *Post hoc* analysis found a significantly greater hemorrhage in subjects that received stimulation compared to subjects that remained unshocked.

The level of hemoglobin-α within the spinal cord tissue did not increased after shock exposure. Using quantitative Western blotting, the level of hemoglobin protein at 15 kDa was analyzed with an ANOVA with shock as the between subjects variable. There were no significant effects, $F < 1.0$, $p > 0.05$ (Figure 8B).

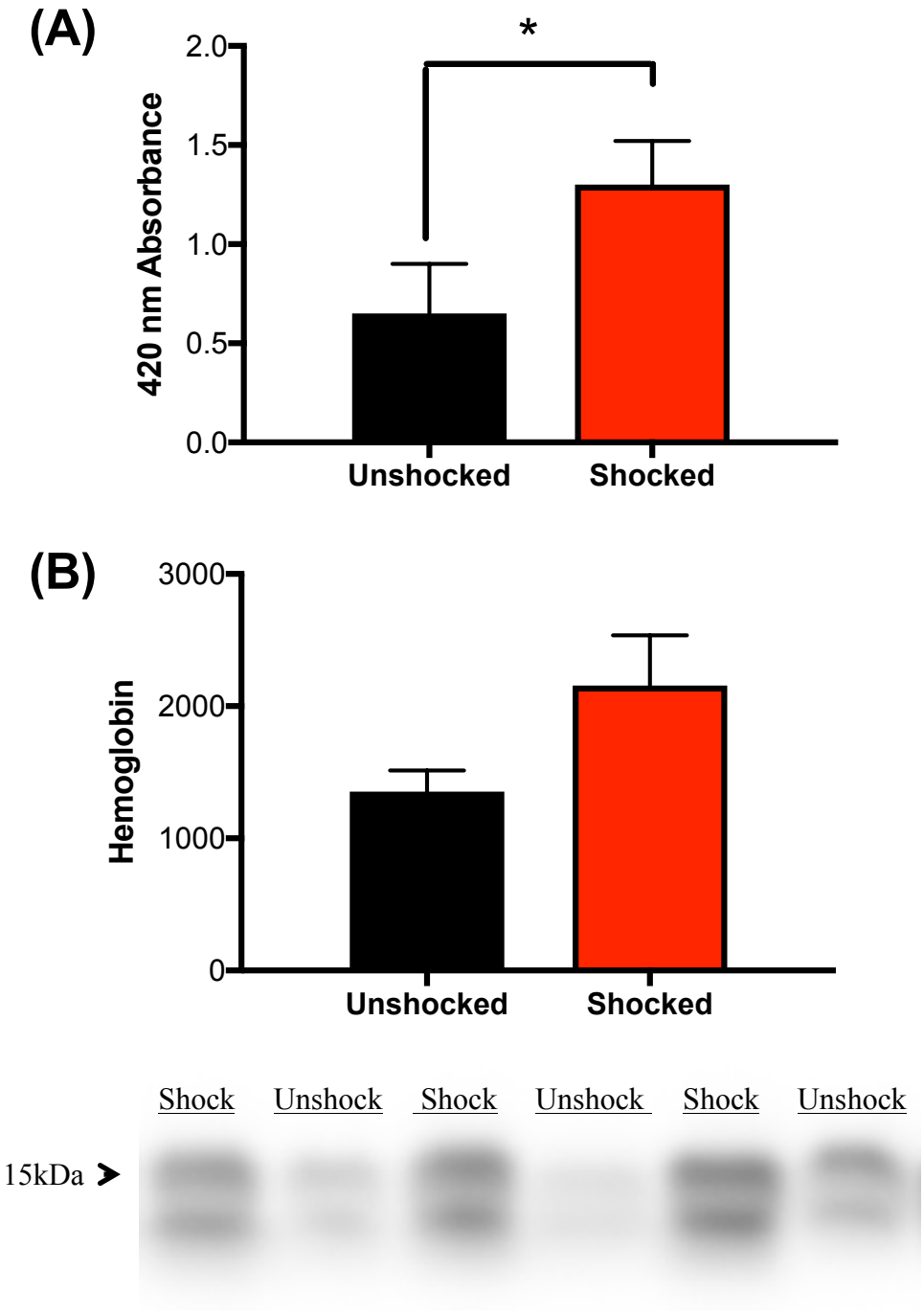


**Figure 8. Hemorrhage three hours after electrical stimulation.** (A) The absorbance at 420 nm was increased thee hours after nociceptive stimulation. (B) The amount of hemoglobin-α did not differ between groups. Error bars represent SEM (n = 8).

Blood pressure measurements were not different between groups prior to treatment. Blood pressure measurements were obtained twenty-four hours after injury, prior to treatment. In addition, mean atrial pressure (MAP) was calculated using the following formula: ((2 x diastolic) + systolic)/3. Pre-treatment values ranged from 107.58 (± 8.8) to 155.94 (± 6.76) millimeter of mercury (mmHg) for systolic, 69.84 (± 7.6) to 77.61 (± 5.05) mmHg for diastolic, 82.11 (± 7.92) to 90.04 (± 5.37) mmHg for MAP, 260.12 (± 27.45) to 294.44 (± 44.62) beats per minute (bpm) for heart rate, and 5.99 (± 1.86) to 6.08 (± 2.57) microliters (μL) per minute for blood flow. Independent ANOVAs revealed no difference between groups on any of these measures, all $F$s < 1.0, $p > 0.05$.

Hypertension was increased after electrical stimulation. Systolic blood pressure was obtained immediately, one, two, and three hours after stimulation to examine how shock treatment affected the development of hypertension. A repeated measures ANCOVA with stimulation as the between subjects variable, baseline systolic as a covariate, and time as a repeated measure revealed a significant main effect of stimulation, $F(1,13) = 30.70$, $p < 0.0001$ (Figure 9A). No other effects were statistically significant, all $F$s < 1.11, $p > 0.05$. *Post hoc* analysis of the main effect showed that subjects receiving electrical stimulation had elevated blood pressure compared to unshocked controls. An identical pattern of results were found for diastolic blood pressure and MAP (data not shown), $F(1,13) > 19.09$, $p < 0.001$.

An elevation in heart rate was seen after electrical stimulation. Heart rates were obtained immediately, one, two, and three hours after stimulation to examine how shock

treatment affected heart rate. A repeated measures ANCOVA with stimulation as the between subjects variable, baseline heart rate as a covariate, and time as a repeated measure, found a significant main effect of stimulation, $F$ (1, 13) = 15.39, $p < 0.01$ (Figure 9B). No other effects were statistically significant, all $F$s < 2.39, $p > 0.05$. *Post hoc* analysis of the main effect showed that subjects receiving electrical stimulation had elevated heart rates compared to unshocked controls.

Cutaneous blood flow, a measure of thermoregulation and vasoconstriction, was increased in subjects receiving electrical stimulation. Blood flow was obtained immediately, one, two, and three hours after stimulation to examine how shock treatment affected blood flow. A repeated measures ANCOVA with stimulation as the between subjects variable, baseline blood flow as a covariate, and time as a repeated measure, found a significant main effect of stimulation, $F$ (1, 13) < 19.03, $p < 0.001$ (Figure 9C). No other effects were statistically significant, all $F$s < 2.40, $p > 0.05$. *Post hoc* analysis of the main effect showed that subjects receiving electrical stimulation had increased blood flow compared to unshocked controls.

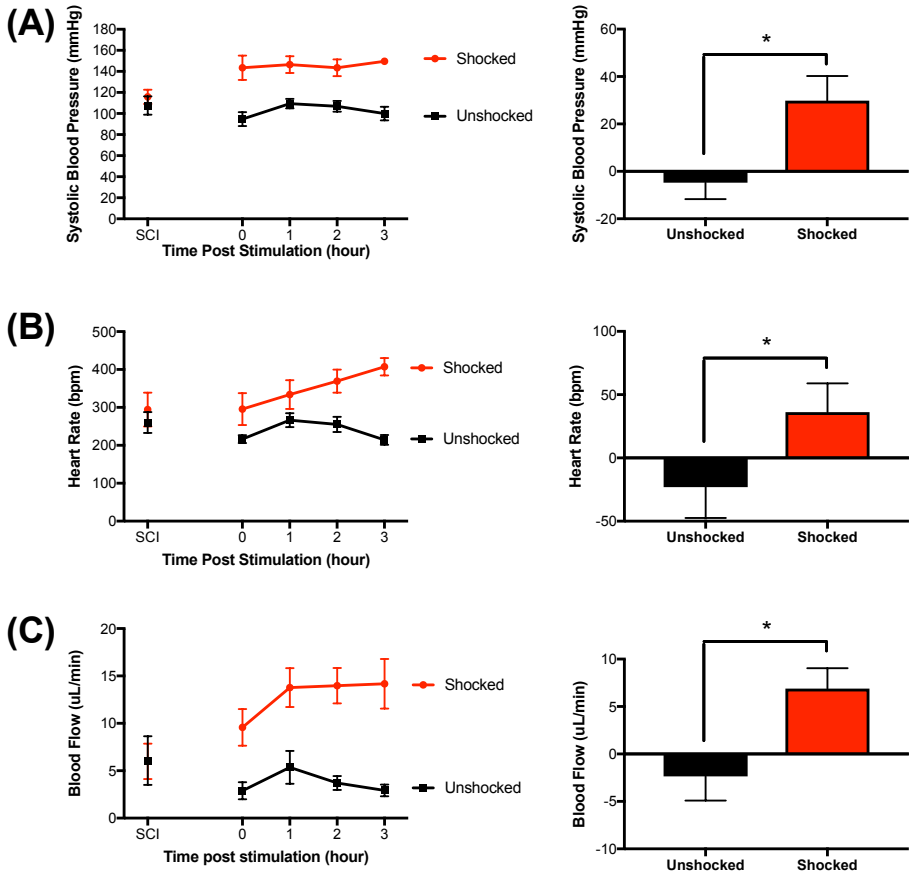


**Figure 9. Blood pressure measurements over the three hours after electrical stimulation.** Electrical stimulation produced a sustained increase in systolic blood pressure, heart rate and blood flow. Error bars represent SEM (n = 8).

Hypertension was correlated with an increase in hemorrhage in subjects receiving shock. Using a Pearson's correlation coefficient, I assessed the relationship between blood pressure and the absorbance of hemoglobin (420 nm) in tissue collected at three hours. A strong positive correlation was found between systolic blood pressure at two and three hours and hemorrhage, $r = 0.71$ and 0.87 (respectively), $p < 0.05$. A similar pattern of results were seen for diastolic blood pressure at three hours and MAP at two and three hours, $r = 0.90$, 0.71, and 0.91(respectively), $p < 0.05$ (data not shown). For unshocked subjects, no correlation between hypertension and hemorrhage was observed, $r < 3.70$, $p > 0.05$. The scatter plot displayed in Figure 10A shows the relationship between systolic blood pressure and hemorrhage at three hours.

An elevation in heart rate was correlated with a decrease in hemorrhage in shocked subjects. Using a Pearson's correlation coefficient, I assessed the relationship between heart rate and the absorbance of hemoglobin (420 nm) in tissue collected at three hours. A strong negative correlation was found between immediate heart rate changes and hemorrhage, $r = -0.81$, $p < 0.05$. In unshocked subjects, there was a significant correlation between heart rate at two and three hours and hemorrhage, this effect was not consistent and did not replicate across studies, $r = 0.77$ and -0.72 (respectively), $p < 0.05$. The scatter plot displayed in Figure 10B shows the relationship between heart rate at three hours and hemorrhage at three hours.

There was no relationship between changes in blood flow and hemorrhage in shocked and unshocked subjects, $r < 0.48$, $p > 0.05$. There was also no relationship between locomotor performance and hemorrhage in shock and unshocked subjects, $r <$

0.52, $p > 0.05$. The scatter plot displayed in Figure 10C and G shows the relationship between blood flow and locomotor scores with hemorrhage at three hours.

An immediate increase in hypertension was correlated with a decrease in locomotor scores in shocked subjects. Using a Pearson's correlation coefficient, I assessed the relationship between blood pressure and locomotor scores. A strong negative correlation was found between immediate changes in diastolic blood pressure and MAP with locomotor scores at one hour, $r = -0.75$ and -0.72 (respectively), $p < 0.05$. For unshocked subjects, no correlation between locomotor scores and hypertension was observed, $r < -0.48$, $p > 0.05$. The scatter plot displayed in Figure 10D shows the relationship between immediate changes in systolic blood pressure and locomotor scores taken at one-hour post injection.

An elevation in heart rate values immediately after treatment correlated with higher locomotor scores in unshocked controls. Using a Pearson's correlation coefficient, I assessed the relationship between heart rate and locomotor scores. A strong positive correlation was found between immediate heart rate changes and locomotor scores at two and three hours post injection, $r = 0.81$ and 0.85 (respectively), $p < 0.05$. For shocked subjects, no correlation between locomotor scores and heart rate was observed, $r < -0.61$, $p > 0.05$. The scatter plot displayed in Figure 10E shows the relationship between immediate changes in heart rate and locomotor scores taken at one-hour post injection.

An immediate increase in blood flow was correlated with a decrease in locomotor scores in shocked subjects. Using a Pearson's correlation coefficient, I

assessed the relationship between blood flow and locomotor scores. A strong negative correlation was found between immediate changes in blood flow and locomotor scores taken at one hour after stimulation, $r = -0.83$, $p < 0.05$. For unshocked subjects, no correlation between locomotor scores and heart rate was observed, $r < -0.54$, $p > 0.05$. The scatter plot displayed in Figure 10F shows the relationship between immediate changes in blood flow and locomotor scores taken at one-hour post injection.

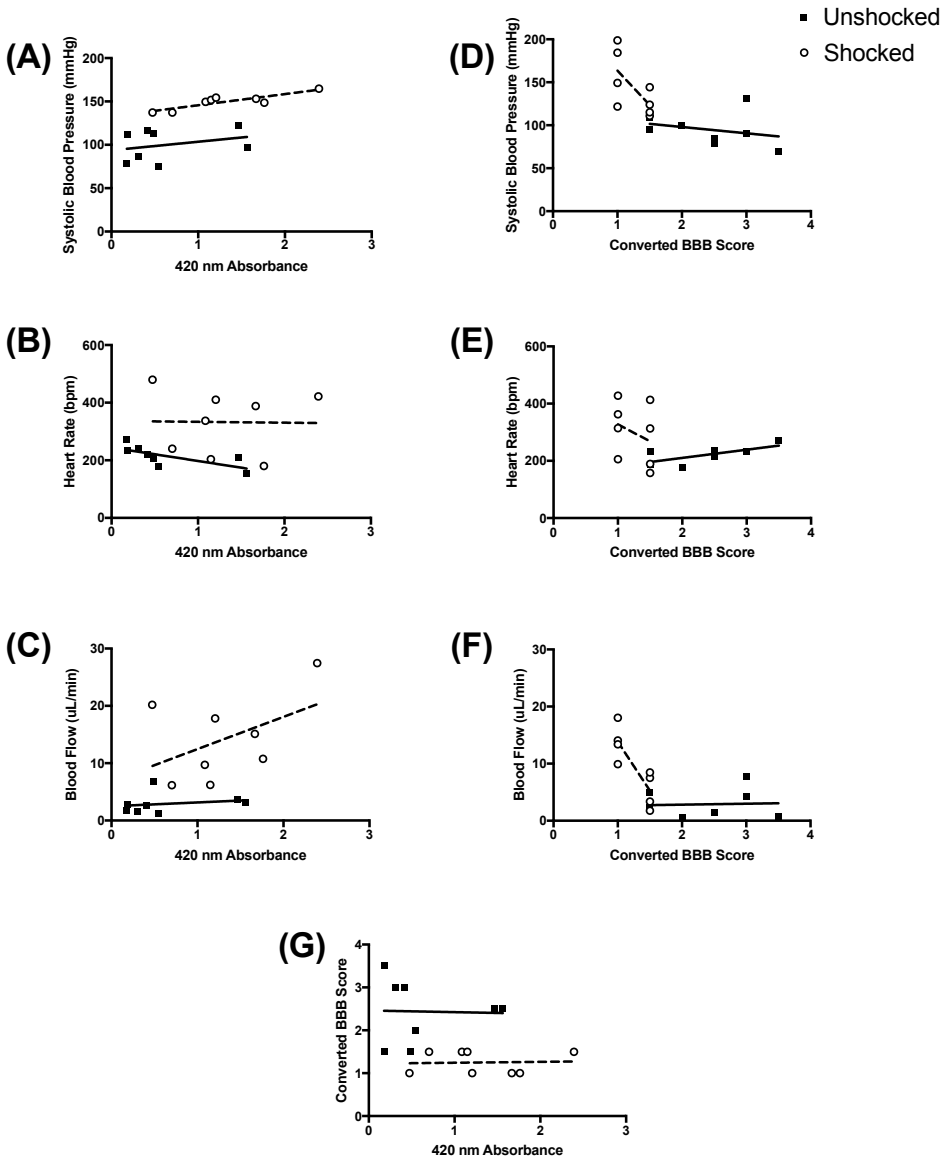


**Figure 10. Scatterplot of groups after electrical stimulation.** (A-C) shows a scatter plot of blood pressure measurements and hemorrhage at three hours. (D-F) show a scatter plot of immediate changes in blood pressure measurements with locomotor scores obtained one hour later. (G) shows a scatter plot of locomotor scores at one hour and hemorrhage at three hours. (n = 8)

*Discussion*

In this study, nociceptive stimulation caused an increase in blood pressure, heart rate, and blood flow. There was also an increase in hemorrhage and decrease in locomotor performance, which replicates previous work (Turtle et al., 2017). Further, an increase in hypertension in shocked subjects was correlated with an increase in hemorrhage at the site of injury. This correlation between hypertension and hemorrhage grew stronger over time.

Hemorrhage was not correlated with locomotor performance over the three hours after shock. There was however a relationship between locomotor performance and blood pressure measurements, with immediate changes correlating with later locomotor scores. This is consistent with previous work showing that blood pressure effects long term recovery (Nielson et al., 2015).

The observation that shock increased heart rate was not surprising because stimulation likely activates sympathetic activity and increases oxygen to muscle. What was interesting was that the only variable correlated with locomotor performance in unshocked controls was an elevation in heart rate. It is likely that animals with a higher, more normal, heart rate have less damage to their spinal cords and thus have better locomotor scores.

In addition to changes in blood pressure and heart rate, there was also a rise in cutaneous blood flow to the tail in shocked subjects. This rise in cutaneous blood flow is common after electrical stimulation and is usually transient (Lynn & Cotsell, 1992). However, given the dysregulation of the autonomic reflexes caused by the injury, this

rise likely puts a strain on the circulatory system as the body struggles to cool itself through vasodilation (Sawka, Latzka, & Pandolf, 1989).

Given that I observed an increases in blood pressure, heart rate, and blood flow, it is likely nociceptive input after injury puts a major strain on the circulatory system, leading to the breakdown of the capillary beds and the infiltration of red blood cells previous observed after shock treatment (Brumley et al., 2016). This in turn may expand the lesion and impair locomotor recovery (Grau et al., 2004). The effect of blood pressure medications on hemorrhage should be examined to see if it can be beneficial to later recovery.

## Experiment 5: Effect of Shock on Blood Pressure at 24 Hours

In the previous study blood pressure, heart rate, and blood flow were elevated for up to three hours after nociceptive stimulation. In this experiment, I examined how long this elevation is maintained after stimulation.

### *Procedure*

Male Sprague Dawley rats (n = 8) received a moderate spinal cord contusion. The next day subjects had their locomotor scores evaluated using the BBB locomotor scale and their baseline blood pressure measurements taken using a non-invasive blood pressure cuff. Subjects were then randomly assigned to electrical stimulation (180 shocks, 1.5mA, 0.2-3.8 second ISI) to the tail or remained unshocked. Groups were balanced using systolic blood pressure and BBB scores obtained 24 hours after injury. BBB scores and blood pressure were monitored at three, six, 12, and 24 hours after electrical stimulation. After obtaining the 24 hours BBB score and blood pressure measurement, subjects were sacrificed and a one-centimeter of spinal tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm) and examined by gel electrophoresis and immunoblotting for hemoglobin.

*Results*

BBB scores did not differ between groups prior to treatment. BBB scores obtained the day after surgery, prior to treatment, ranged from 3.00 (± 0.39) to 3.44 (± 0.52). An ANOVA found no differences between groups, $F$ (1, 14) < 1.0, $p$ > 0.05.

BBB scores were obtained three, six, 12, and 24 hours after stimulation to examine whether shock affected locomotor scores. Locomotor scores did not differ across groups six hours after electrical stimulation. A repeated measures ANCOVA with shock as the between subjects variable, baseline BBB scores as the covariate, and time as the repeated measure revealed no significant effects, $F$ (1, 13) < 2.47, $p$ > 0.05 (Figure 11). Preplanned analysis of the three hours time point using an ANCOVA revealed a significant decrease in locomotor scores in shocked subjects, $F$ (1, 13) = 8.80, $p$ < 0.05 (Figure 11).

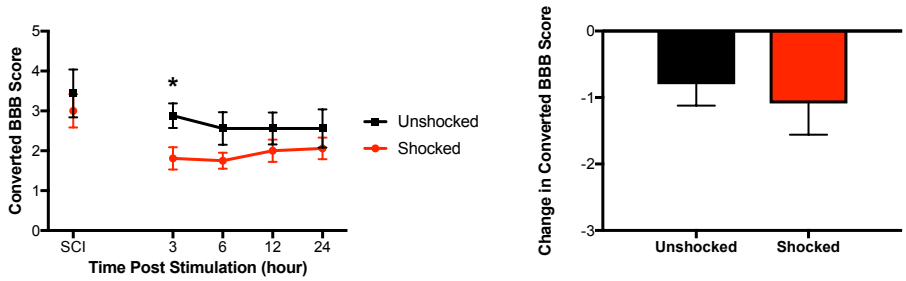


**Figure 11. Locomotor performance over the 24 hours after electrical stimulation**. BBB locomotor scores at three hours were decreased in shocked subjects. Error bars represent SEM (n = 8).

The increase in hemorrhage was no longer evident 24 hours after injury in shocked subjects. The magnitude of the peak in absorbance at 420 nm was analyzed in tissue collected 24 hours after stimulation using an ANOVA with shock condition as the between subjects variable. Analysis revealed no significant differences, $F < 1.6$, $p > 0.05$ (Figure 12A).

The level of hemoglobin-α within the spinal cord tissue was not different between groups. Using quantitative Western blotting, the level of hemoglobin protein at 15 kDa was analyzed with an ANOVA with shock as the between subjects variable. The overall analysis found no significant differences between groups, $F < 1.0$, $p > 0.05$ (Figure 12B).

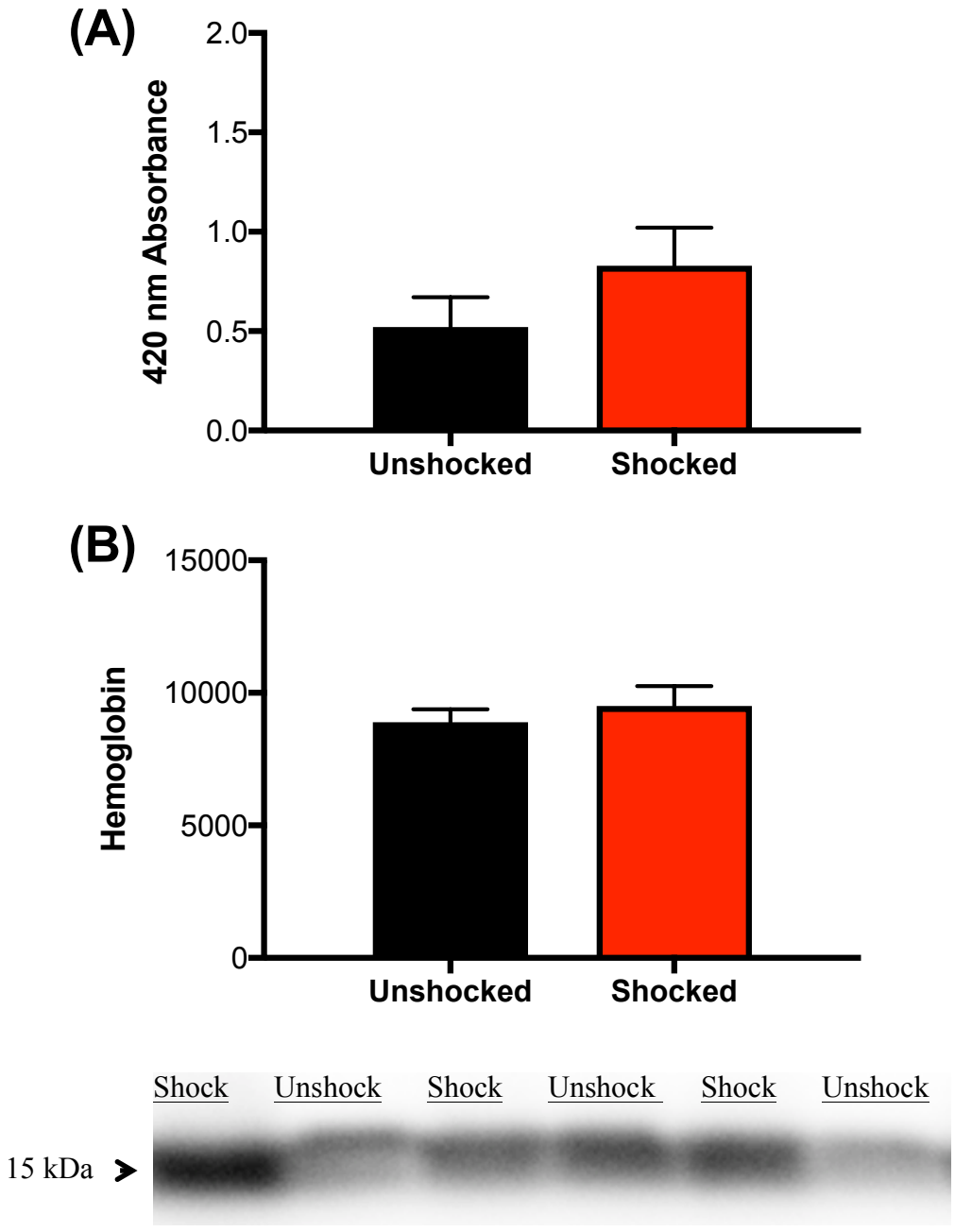


**Figure 12. Hemorrhage 24 hours after electrical stimulation**. (A) The absorbance at 420 nm did not differ across groups. (B) The amount of hemoglobin-α did not differ between groups. Error bars represent SEM (n = 8).

Blood pressure measurements were not different between groups prior to treatment. Pre-treatment values ranged from 111.67 (± 9.2) to 115.08 (± 7.37) mmHg for systolic, 76.83 (± 5.51) to 77.74 (± 6.23) mmHg for diastolic, 88.71 (± 7.17) to 89.28 (± 5.98) mmHg for MAP, 233.44 (± 22.41) to 273.38 (± 13.47) bpm for heart rate, and 6.05 (± 1.79) to 4.34 (± 1.10) μL per minute for blood flow. Independent ANOVAs revealed no difference between groups on any of these measures, all $F$s < 3.38, $p > 0.05$.

The amount of hypertension was not different between groups beginning six hours after stimulation. Blood pressure measurements were obtained at three, six, 12, and 24 hours after stimulation to examine the effect of stimulation on systolic blood pressure. A repeated measures ANCOVA with stimulation as the between subjects variable, baseline as a covariate, and time as a repeated measure found no significant effects, all $F$s < 1.90, $p > 0.05$ (Figure 13A). Likewise, analysis of diastolic blood pressure and MAP also showed no significant change, all $F$s < 2.74, $p > 0.05$ (data not shown). Preplanned analysis of the three hours time point using an ANCOVA revealed a significant increase in systolic blood pressure, diastolic blood pressure, and MAP in shocked subjects, $F$ (1, 13) > 9.0, $p < 0.01$ (Figure 13B).

Subjects continued to show elevated heart rates for up to 24 hours after nociceptive stimulation. Heart rates were obtained three, six, 12, and 24 hours after stimulation. A repeated measures ANCOVA with stimulation as the between subjects variable, baseline heart rate as a covariate, and time as a repeated measure found a significant main effect of stimulation, $F$ (1, 13) = 9.05, $p < 0.01$ (Figure 13C). No other

effects were significant, $F$ (1, 13) < 1.0, $p$ > 0.01. *Post hoc* analysis revealed a significant elevation in heart rate for subjects receiving stimulation.

An increase in blood flow was seen 24 hours after stimulation. Blood flow measurements were obtained three, six, 12, and 24 hours after stimulation. A repeated measures ANCOVA with stimulation as the between subjects variable, baseline blood flow as a covariate, and time as a repeated measure found a significant main effect of stimulation, $F$ (1, 14) = 5.34, $p$ < 0.05 (Figure 13). No other effects were statistically significant, $F$ (1, 14) < 1.0, $p$ > 0.05. *Post hoc* analysis revealed an increase in blood flow in subject receiving electrical stimulation.

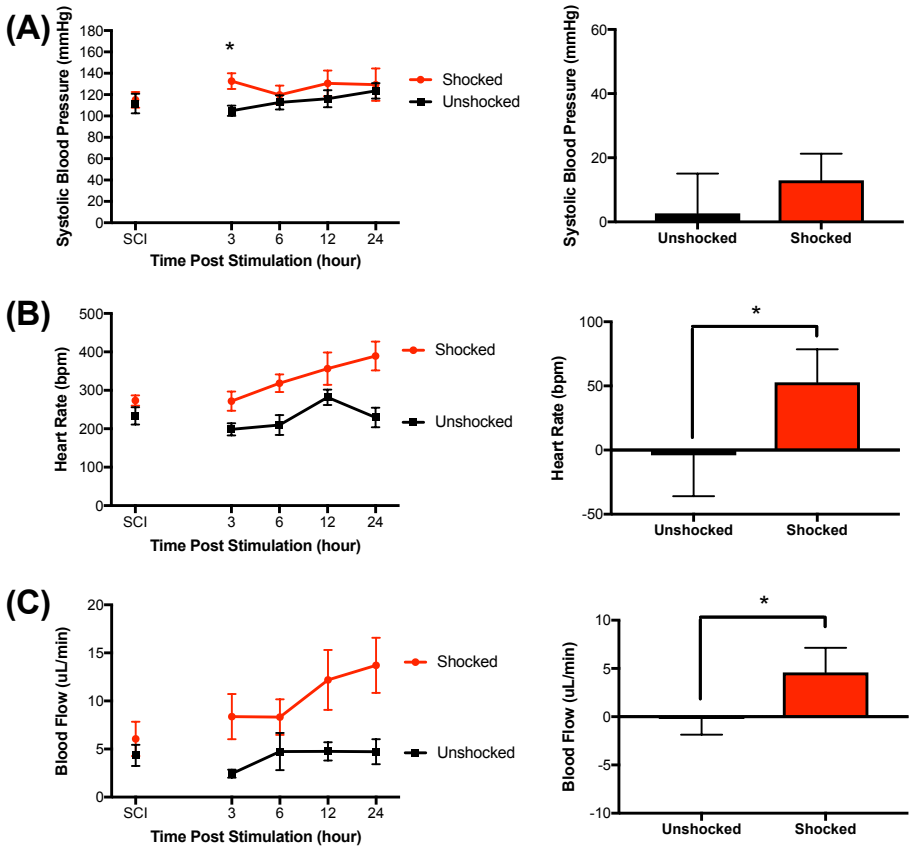


**Figure 13. Blood pressure measurements over the 24 hours after electrical stimulation.** Heart rate and blood flow continued to be increased in shocked subjects 24 hours after electrical stimulation. Systolic blood pressure was only elevated at the three-hour time point. Error bars represent SEM (n = 8).

In shocked subjects, an increase in hypertension was correlated with an increase in the amount of hemorrhage. Using a Pearson's correlation, I assessed the relationship between blood pressure and absorbance of hemoglobin (420 nm) in tissue collected at 24 hours. A strong positive correlation was found between systolic blood pressure at 12 hours and hemorrhage, $r = 0.80$, $p < 0.05$. Similarly, a strong correlation was found between diastolic blood pressure and MAP measurements obtained 12 hours after stimulation and hemorrhage, $r = 0.74$, and 0.77 (respectively), $p < 0.05$. There was no relationship between hypertension and hemorrhage in unshocked subjects, $r < 0.68$, $p > 0.05$. The scatter plot displayed in Figure 14A shows the relationship between systolic blood pressure at 12 hours and hemorrhage at 24 hours.

In shocked subjects, an elevated heart rate was correlated with an increase in hemorrhage. Using a Pearson's correlation, I assessed the relationship between heart rate and absorbance of hemoglobin (420 nm) in tissue collected at 24 hours. A strong positive correlation was found between heart rate at three and 12 hours and hemorrhage, $r = 0.74$ and 0.76 (respectively), $p < 0.05$. There was no relationship between hemorrhage and heart rate in unshocked subjects, $r < 0.35$, $p > 0.05$. The scatter plot displayed in Figure 14B shows the relationship between heart rate at 12 hours and hemorrhage at 24 hours.

An increase in blood flow was correlated with hemorrhage. Using a Pearson's correlation, I assessed the relationship between blood flow and absorbance of hemoglobin (420 nm) in tissue collected at 24 hours. A strong positive correlation was found between blood flow at 12 hours and hemorrhage in both shocked and unshocked

subjects, $r = 0.80$ and 0.82 (respectively), $p < 0.05$. Additionally, blood flow at six hours was correlated with hemorrhage in shocked subjects, $r = 0.82$, $p < 0.05$. The scatter plot displayed in Figure 14C shows the relationship between heart rate at 12 hours and hemorrhage at 24 hours.

For shocked subjects, lower locomotor scores correlated with higher hemorrhage. Using a Pearson's correlation, I assessed the relationship between locomotor scores and absorbance of hemoglobin (420 nm) in tissue collected at 24 hours. A strong negative correlation was found between locomotor scores obtained at three, 12, and 24 hours and hemorrhage, $r = -0.74$, -0.75, and -0.72 (respectively), $p < 0.05$. There was no relationship between hemorrhage and locomotor scores in unshocked subjects, $r > -0.46$, $p > 0.05$. The scatter plot displayed in Figure 14G shows the relationship between locomotor scores at three hours and hemorrhage at 24 hours.

As blood pressure measurements at multiple time points were highly correlated with locomotor scores at multiple times points, I simplified the analysis by focusing on the 12 hours time point for locomotor performance because it was highly correlated with hemorrhage in the above section.

Hypertension was correlated with lower BBB scores. Using a Pearson's correlation, I assessed the relationship between blood pressure and 12 hour locomotor scores. In shocked subjects, a strong negative correlation was found between systolic blood pressure at three, six, and, 12 hours and locomotor scores at 12 hours, $r = -0.82$, -0.80, and -0.78 (respectively), $p < 0.05$. Similarly, diastolic blood pressure and MAP at 12 hours correlated with locomotor scores at 12 hours, $r = -0.84$ and -0.72 (respectively),

$p < 0.05$. MAP at six hours was also correlated with locomotor scores at 12 hours, $r = -0.72$, $p < 0.05$. There was no relationship between hypertension and 12 hour locomotor scores in unshocked subjects, $r > -0.57$, $p > 0.05$. The scatter plot displayed in Figure 14D shows the relationship between systolic blood pressure and locomotor scores at 12 hours.

An elevated heart rate was correlated with lower locomotor scores. Using a Pearson's correlation, I assessed the relationship between heart rate and 12 hour locomotor scores. In shocked subjects, a strong negative correlation was found between heart rate at 12 hours and locomotor scores at 12 hours, $r = -0.84$, $p < 0.05$. Similarly in unshocked subjects, heart rate at 12 hours was correlated with locomotor 12, $r = -0.79$, $p < 0.05$. The scatter plot displayed in Figure 14E shows the relationship between heart rate and locomotor scores at 12 hours.

An increase in cutaneous blood flow was correlated with lower locomotor scores in shocked subjects. Using a Pearson's correlation, I assessed the relationship between blood flow and 12 hour locomotor scores. In shocked animals, a strong negative correlation was found between blood flow at six, 12, and 24 hours and locomotor scores at 12 hours, $r = -0.82$, -0.75, and –0.73, respectively), $p < 0.05$. There was no relationship between blood flow and 12 hour locomotor scores in unshocked subjects, $r > -0.57$, $p > 0.05$. The scatter plot displayed in Figure 14F shows the relationship between blood flow and locomotor scores at 12 hours.

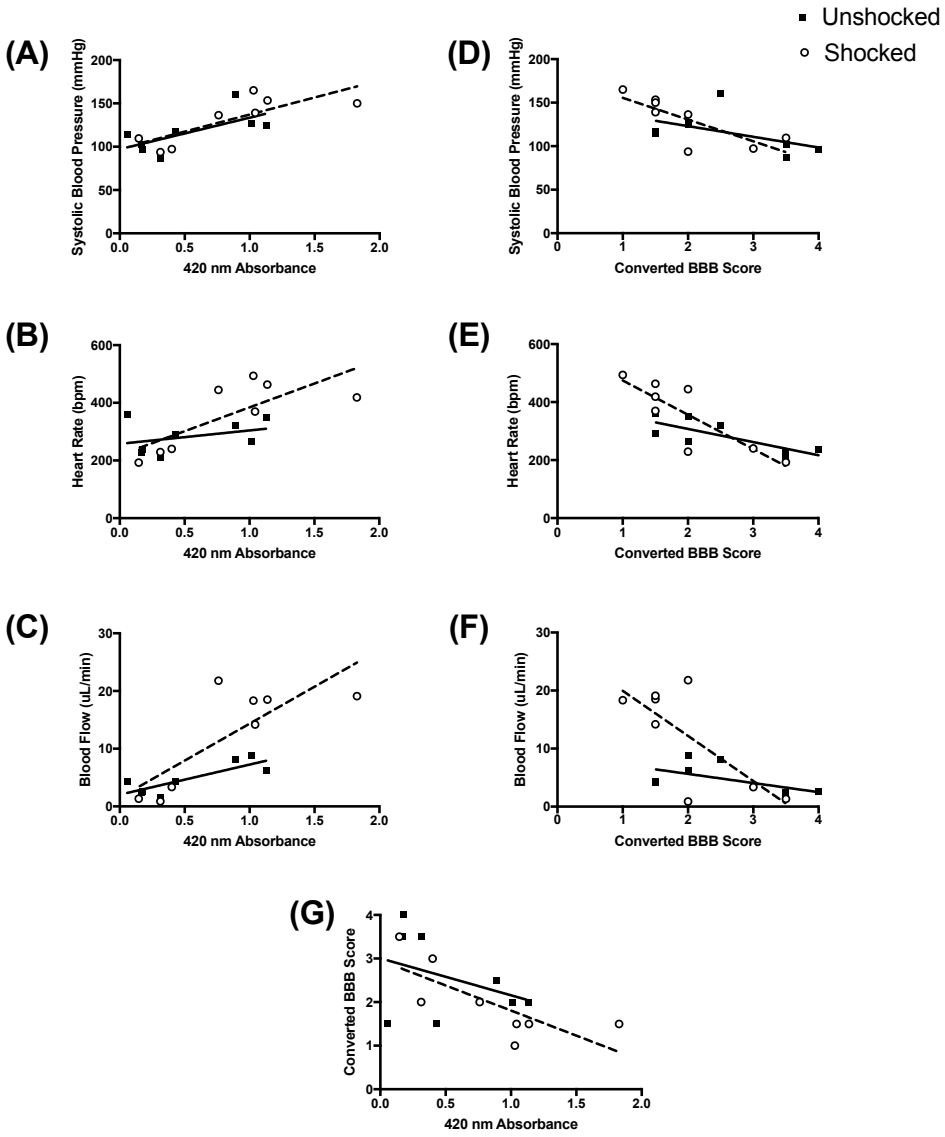


**Figure 14. Scatterplot of groups a day after nociceptive stimulation.** (A-C) shows a scatter plot of blood pressure measurements at 12 hours and hemorrhage at 24 hours. (D-F) show a scatter plot of blood pressure measurements and locomotor scores obtained 12 hours after injection. (G) shows a scatter plot of locomotor scores at three hours and hemorrhage at 24 hours. (n = 8)

*Discussion*

In this study, heart rate and blood flow remained elevated 24 hours after electrical stimulation in shocked subjects. In uninjured people, individuals electrical stimulation produces a transient rise in heart rate and blood flow (Kang & Hyong, 2014). Also, it is typically to see a rebound in heart rate rise after a fall in blood pressure. What was interesting here was how long heart rate and blood flow were sustained after electrical shock. One possible reason for this sustained increase could be due to an increase in the pro-inflammatory cytokine TNFα. Research has shown that noxious input after SCI can increase the expression of TNFα resulting in the activation of cell death pathways (Garraway et al., 2014). Further, TNFα increased heart rate and reduces the contractibility strength of the heart when injected. Interestingly, TNFα was also synthesized in the heart in response to pathophysiological conditions such as acute hemodynamic overloading, ischemia, and sepsis (Rathi, Xu, & Dhalla, 2002). While at low levels TNFα was thought to be cardioprotective, at high levels of circulating TNFα was pathogenic to the heart. Another possibility was that there was a form of conditioned fear that increases. Given that rats were shocked in tubes, there could be a generalization of condition fear to the blood pressure tubes. This seems unlikely, however, because rats were acclimated in the blood pressure tubes before the day of testing and the tubes were very different in color and form.

I observed an increase in blood pressure and a decrease in locomotor performance at three hours in shocked subjects, replicating the previous study. Additionally, this follows the timeline of peak hemorrhage observed in a previous study.

This supports the idea that early time period after pain input is important targets for therapy.

Hemorrhage, as measured by absorbance at 420 nm, was not elevated compared control 24 hours after simulation. This replicates a previous study showing that hemorrhage was elevated in shocked subjects at one and three hour after simulation, peaking at three hours, but gone by 24 hours after stimulation (Turtle et al., 2015).

While I did not see a correlation between hemorrhage and locomotor scores in the previous study, I found a strong correlation between locomotor scores and hemorrhage in the current study. This suggests that the effects of nociceptive input on locomotor performance took time to develop.

# CHAPTER V

# THE EFFECT OF CAPSAICIN ON BLOOD PRESSURE

In order to better understand how nociceptive activation affects SCI recovery, research has assessed the effect of peripheral irritants, such as capsaicin (Turtle et al., 2015) . Capsaicin, the active ingredient in chili peppers, binds to TRPV1 channels (Szallasi & Blumberg, 1999). Unlike electrical stimulation of c-fibers, capsaicin produces a prolonged burning pain sensation that is accompanied by inflammation. Previous research using capsaicin after SCI has found that a single injection of capsaicin the day after injury produced a robust locomotor deficit and an increase in pain sensation (Garraway et al., 2014; Hook, Huie, & Grau, 2008). Examination of the spinal cord tissue shortly after injury found an increase in cell death markers, inflammatory cytokines, and hemorrhage (Turtle et al., 2015) .

The current set of experiments explored whether the expansion of the hemorrhage and impaired locomotor performance seen after capsaicin were due in part to changes in blood pressure. Experiment 6 examined whether blood pressure and hemorrhage were increased with in the first three hours after injection. Experiment 7 examined whether blood pressure and hemorrhage were increased 24 hours after injection of capsaicin.

## Experiment 6: Effect of Capsaicin on Blood Pressure at Three Hours

In the previous study I found that blood pressure, heart rate, and blood flow were elevated for up to three hours after nociceptive stimulation. In this experiment I examined whether capsaicin produced an increase in blood pressure within three hours after injection.

### *Procedure*

Twenty-four hours after receiving a moderate spinal cord contusion at T12, male Sprague Dawley rats (n = 8) had their locomotor scores evaluated using the BBB locomotor scale and their baseline blood pressure measurements taken using a non-invasive blood pressure cuff. Subjects were then randomly assigned to receive an intradermal injection of capsaicin (3%) or vehicle. Groups were balanced using baseline systolic blood pressure and BBB scores. BBB scores and blood pressure were monitored immediately, one, two, and three hours after injection. After obtaining the three hours BBB score and blood pressure measurement, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm) and examined by gel electrophoresis and immunoblotting for hemoglobin.

*Results*

BBB score did not differ between groups prior to treatment. Baseline BBB scores taken 24 hour after spinal cord injury ranged from 3.50 (± 0.63) to 3.75 (± 0.70). An ANOVA revealed no difference between groups, $F$ (1, 14) < 1.0, $p > 0.05$.

Locomotor scores were significantly lower after capsaicin treatment. To examine whether capsaicin affected locomotor scores over time, a repeated measures ANCOVA was used with injection as the between subjects variable, baseline BBB scores as the covariate, and time as the repeated measure. Overall analysis revealed a main effect of injection, $F$ (1, 13) = 9.43, $p < 0.01$ (Figure 15). No other effects were statistically significant, all $F$s < 1.0, $p > 0.05$. *Post hoc* analysis showed a reduction in locomotor scores in subjects receiving capsaicin.

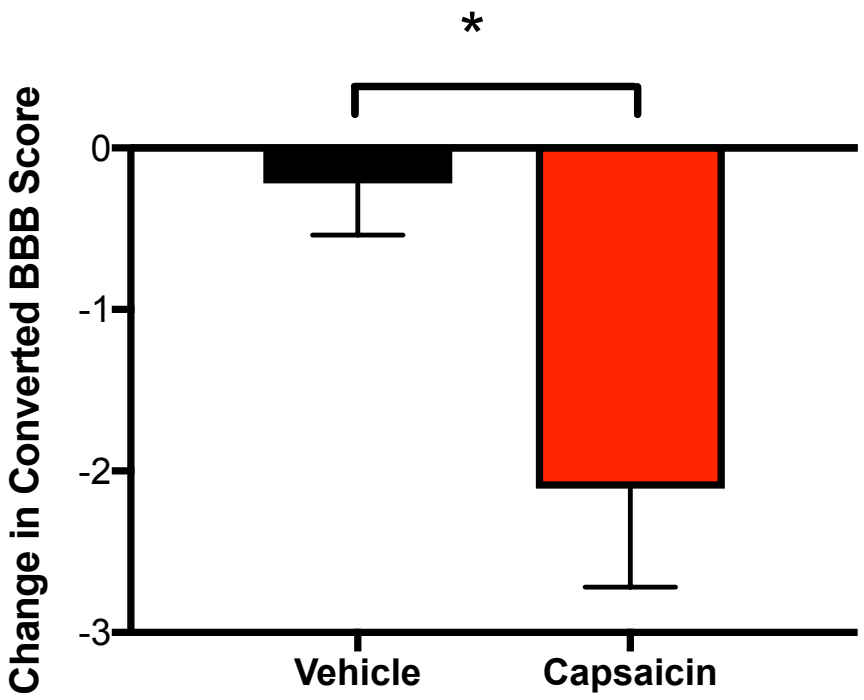


**Figure 15. Locomotor performance over the three hours after capsaicin injection**. BBB locomotor scores taken during the three hours post stimulation period decreased in subjects receiving capsaicin. Error bars represent SEM (n = 8).

Capsaicin treatment increased the expansion of the hemorrhage three hours after injection. The magnitude of the peak in absorbance at 420 nm was analyzed using an ANOVA with injection as the between subjects variable. A significant main effect of injection was observed, $F$ (1,14) = 13.186, $p < 0.05$ (Figure 16A). Subsequent analysis found that subjects treated with capsaicin showed an increase in hemorrhage.

The level of hemoglobin-α within the spinal cord tissue was not different between groups. Using quantitative Western blotting, the level of hemoglobin protein at 15 kDa was analyzed with an ANOVA with injection as the between subjects variable. The overall analysis found no significant differences between groups, $F$ (1,14) = 3.79, $p$ = 0.07 (Figure 16B).

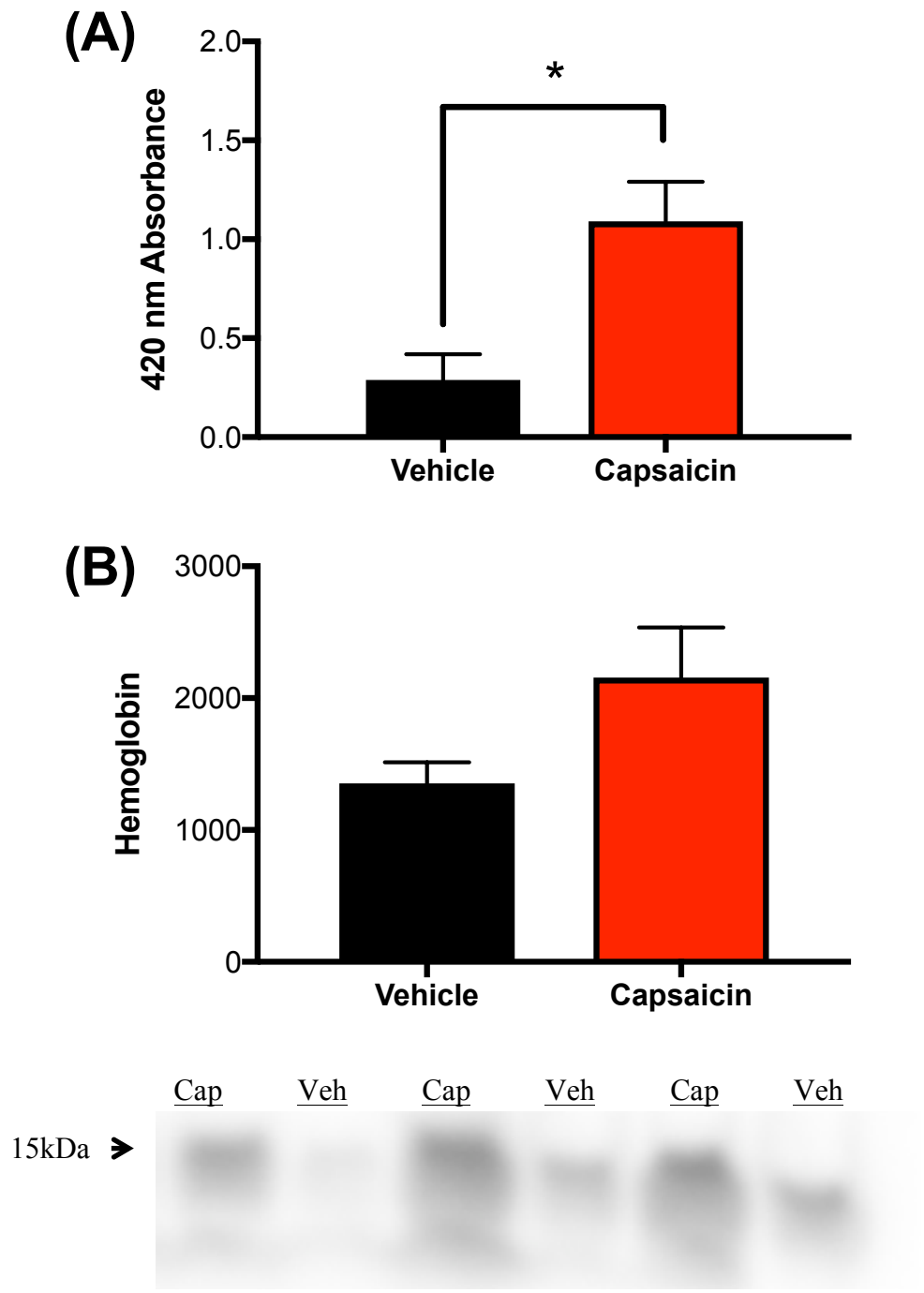


**Figure 16. Hemorrhage three hours after capsaicin injection.** The absorbance at 420 nm taken at three hours was increased after capsaicin treatment. Error bars represent SEM (n = 8).

Blood pressure measurements did not differ between groups prior to treatment. Pre-treatment values ranged from 97.59 (± 8.91) to 104.04 (± 9.11) mmHg for systolic, 64.75 (± 5.94) to 69.61 (± 5.01) mmHg for diastolic, 75.38 (± 6.76) to 80.78 (± 6.29) mmHg for MAP, 253.13 (± 16.97) to 253.32 (± 24.62) bpm for heart rate, and 2.64 (± 0.73) to 3.58 (± 1.16) μL per minute for blood flow. Independent ANOVAs revealed no difference between groups on any of these measures, all $F$s < 1.0, $p > 0.05$

Capsaicin treatment has no effect on hypertension after SCI. Blood pressure measurements were obtained immediately, one, two, and three hours after stimulation to examine the effect of injection on systolic blood pressure. A repeated measures ANCOVA with stimulation as the between subjects variable, baseline systolic as a covariate, and time as a repeated measure found no significant effects, all $Fs < 1.0$, $p > 0.05$ (Figure 17A). Analysis of diastolic blood pressure and MAP showed similar null effects, all $Fs < 1.0$, $p > 0.05$ (data not shown).

Heart rate was elevated three hours after capsaicin treatment. Heart rate was obtained immediately, one, two, and three hours after stimulation. A repeated measures ANCOVA with injection as the between subjects variable, baseline as the covariate, and time as a repeated measure, found a significant interaction between injection and time, $F(3, 39) = 4.00$, $p < 0.05$ (Figure 17B). No other effects were statistically significant, all $F$s < 1.0, $p > 0.05$. *Post hoc* analysis of the interaction revealed an increase in heart rate beginning three hours after capsaicin treatment compared to vehicle controls.

Blood flow was increased after capsaicin treatment. Blood flow measurements were obtained immediately after stimulation and at one, two, and three hours after

stimulation. A repeated measures ANCOVA with injection as the between subjects variable, baseline as the covariate, and time as a repeated measure found a significant main effect of injection, $F$ (1, 13) = 5.00, $p < 0.05$ (Figure 17C). No other effects were statistically significant, all $F$s < 1.0, $p > 0.05$. *Post hoc* analysis of the main effect showed that subjects receiving capsaicin treatment had increased blood flow compared to vehicle controls.

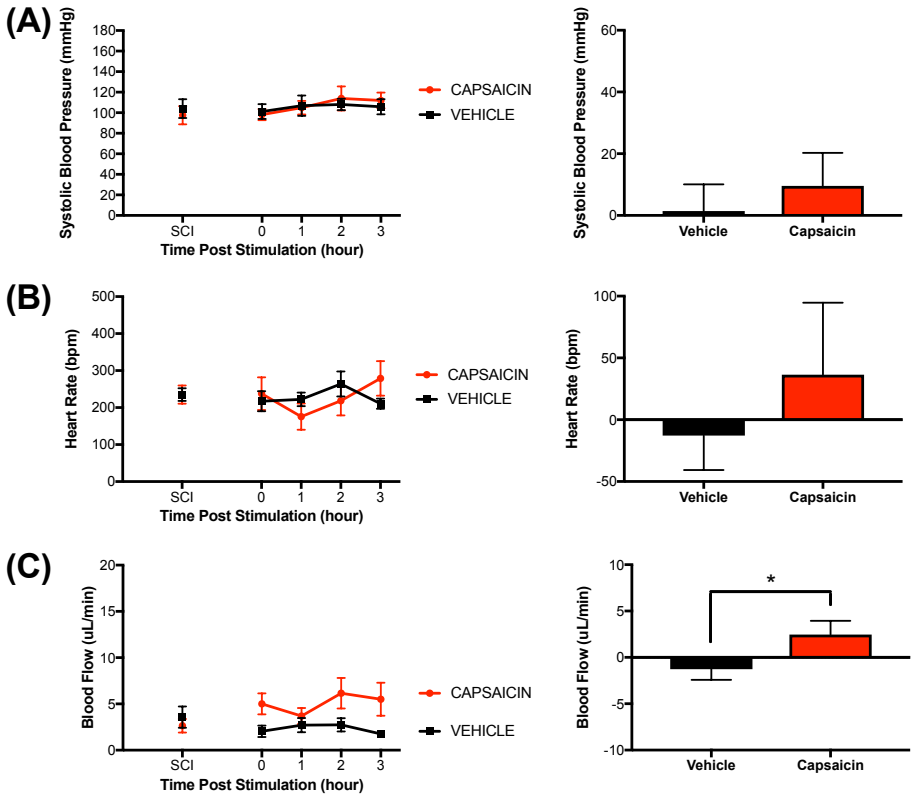


**Figure 17. Blood pressure measurements over the three hours after capsaicin injection.** Capsaicin treatment did not increase systolic blood pressure, but increased heart rate and blood flow. Error bars represent SEM (n = 8).

Hypertension was not correlated with hemorrhage in capsaicin and vehicle subjects, $r < 0.60$, $p > 0.05$ (Figure 18A)

An elevation in heart rate was correlated with a decrease in hemorrhage in capsaicin treated subjects. Using a Pearson's correlation coefficient, I assessed the relationship between heart rate and the absorbance of hemoglobin (420 nm) in tissue collected at three hours. A strong negative correlation was found between heart rate immediately, one, and two hours after injection and hemorrhage, $r = -0.74$, -0.73, and -0.73 (respectively), $p < 0.05$. Heart rate was not correlated with hemorrhage in vehicle controls, $r < 0.56$, $p > 0.05$. The scatter plot displayed in Figure 18B shows the relationship between immediate changes in heart rate and hemorrhage at three hours.

An increase in blood flow was correlated with a decrease in hemorrhage. Using a Pearson's correlation coefficient, I assessed the relationship between blood flow and the absorbance of hemoglobin (420 nm) in tissue collected at three hours. A strong negative correlation was found between immediate changes in blood flow and hemorrhage for capsaicin and vehicle groups, $r = -0.79$ and -0.73, $p < 0.05$. The scatter plot displayed in Figure 18C shows the relationship between immediate changes in blood flow and hemorrhage at three hours.

Lower locomotor score were correlated with an increase in hemorrhage in capsaicin treated subjects. Using a Pearson's correlation coefficient, I assessed the relationship between locomotor performance and the absorbance of hemoglobin (420 nm) in tissue collected at three hours. A strong negative correlation was found between immediate changes in locomotor scores and hemorrhage, $r = -0.78$, $p < 0.05$. Locomotor

performance was not correlated with hemorrhage in vehicle controls, $r < -0.61$, $p > 0.05$. The scatter plot displayed in Figure 18G shows the relationship between immediate changes in locomotor scores and hemorrhage at three hours.

As blood pressure measurements at multiple time points were highly correlated with locomotor scores at multiple times points, I simplified our analysis by focusing on the immediate time point for locomotor performance because it was highly correlated with hemorrhage in the above section.

Hypertension was not correlated with immediate changes in locomotor performance in capsaicin and vehicle subjects, $r < -0.63$, $p > 0.05$ (Figure 18D).

Elevated heart rate was correlated with higher locomotor scores in capsaicin treated subjects. Using a Pearson's correlation coefficient, I assessed the relationship between heart rate and locomotor performance. A strong positive correlation was found between immediate changes in locomotor scores and heart rate immediately, one, and two hours after injection, $r = 0.89$, 0.88, and 0.81 (respectively), $p < 0.05$. Immediate changes in locomotor performance did not correlated with heart rate in vehicle subjects, $r < 0.41$, $p > 0.05$. The scatter plot displayed in Figure 18E shows the relationship between immediate changes in heart rate and locomotor scores.

An increase in blood flow was correlated with higher locomotor recover in capsaicin treated subjects. Using a Pearson's correlation coefficient, I assessed the relationship between blood flow and locomotor performance. A strong positive correlation was found between immediate changes in blood flow and immediate changes in locomotor scores, $r = 0.90$, $p < 0.05$. Immediate changes in locomotor performance

was not correlated to blood flow in vehicle subjects, $r < -0.61$, $p > 0.05$. The scatter plot displayed in Figure 18F shows the relationship between immediate changes in blood flow rate and locomotor scores.

*Discussion*

Capsaicin treatment increased absorbance in the region associate with hemoglobin three hours after injection. This replicated the previous studies, showing that noxious input produced an increase in hemorrhage when given soon after injury. It is also consistent with data showing that capsaicin treatment decreased locomotor performance.

Interestingly, capsaicin did not induce hypertension. This calls into question the idea that hypertension is necessarily linked to nociception-induced hemorrhage. What is less clear is whether this was observed because shock and capsaicin induced hemorrhage in distinct ways or because the hypertension observed after shock treatment was unrelated to its effect on tissue sparing. Further work is needed to address these issues.

While capsaicin did not increase blood pressure, it did cause a transient increase in heart rate at three hours and a sustained increase in blood flow. As mentioned previous, an increase in inflammation or stress may underlie this increase in heart rate and blood flow.

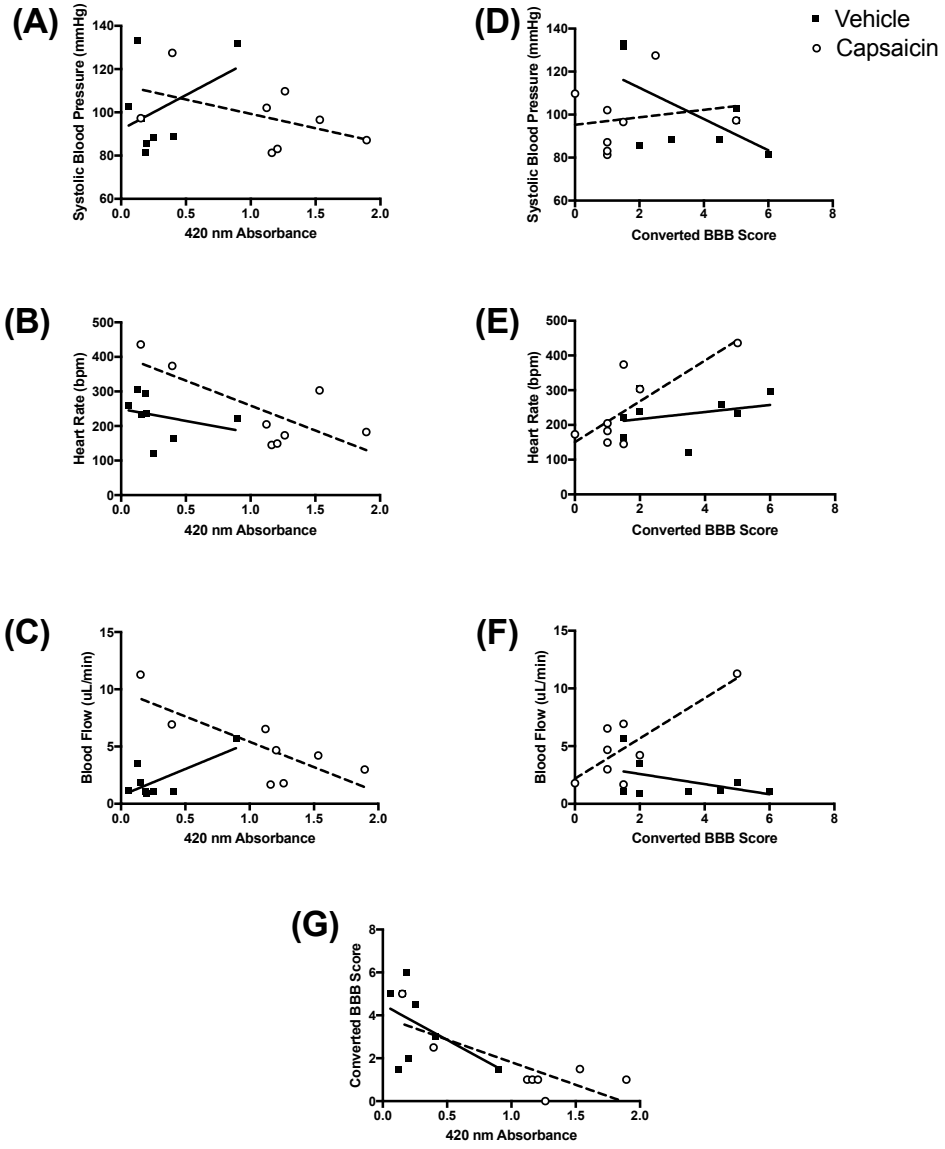


**Figure 18. Scatterplot of groups after capsaicin injection.** Graphs A-C shows a scatter plot of the immediate blood pressure measurements and hemorrhage at three hours. Graphs D-F show a scatter plot of immediate changes in blood pressure measurements and locomotor scores. (n = 8)

## Experiment 7: Effect of Capsaicin on Blood Pressure at 24 Hours

In the previous study, capsaicin caused a sustained increased in blood flow and a transient increase in heart rate at three hours. To examine this effect over time, and evaluate the possibility that capsaicin treatment may induce hypertension at later time points, I assessed blood pressure and hemorrhage over a longer interval (24 hours).

### *Procedure*

Twenty-four hours after receiving a moderate spinal cord contusion at T12, male Sprague Dawley rats (n = 8) had their locomotor scores evaluated using the BBB locomotor scale and their baseline blood pressure measurements taken using a non-invasive blood pressure cuff. Subjects were then randomly assigned to receive an intradermal injection of capsaicin (3%) or vehicle. Groups were balanced using baseline systolic blood pressure and BBB scores. BBB scores and blood pressure were obtained at three, six, 12, and 24 hours after injection. After obtaining the 24 hours BBB score and blood pressure measurement, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm) and examined by gel electrophoresis and immunoblotting for hemoglobin.

*Results*

BBB scores did not differ between groups prior to treatment. BBB scores obtained the day after surgery, prior to treatment, ranged from 3.69 (± 0.6) to 3.88 (± 0.39). An ANOVA revealed no difference between groups, $F(1, 14) < 1.0$, $p > 0.05$.

Locomotor performance was reduced in capsaicin treated subjects. BBB scores were obtained three, six, 12, and 24 hours after injection to examine whether capsaicin affected locomotor scores. A repeated measures ANCOVA was used with injection as the between subjects variable, baseline as the covariate, and time as the repeated measure. Overall analysis revealed a main effect of injection, $F(1, 13) = 12.839$, $p < 0.01$. No other effects were statistically significant, $F(3, 39) < 1.0$, $p > 0.05$. *Post hoc* analysis revealed a significant decrease in locomotor performance in animals injected with capsaicin (Figure 19).

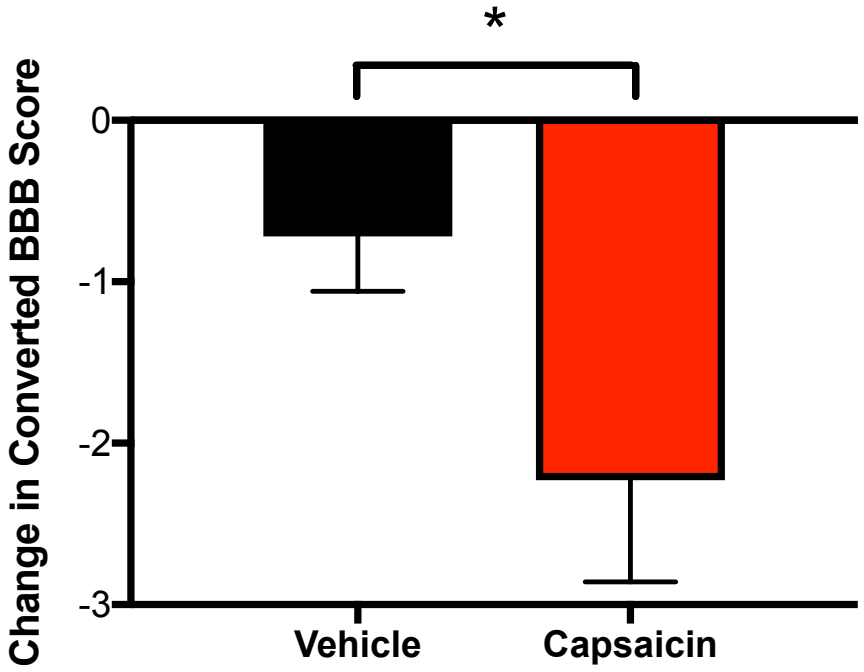


**Figure 19. Locomotor performance over the 24 hours after capsaicin injection.** BBB locomotor scores were reduced in subjects treated with capsaicin. Error bars represent SEM (n = 8)

Capsaicin increased the amount of hemorrhage in the spinal cord 24 hours after injection. The magnitude of the peak in absorbance at 420 nm was analyzed using an ANOVA with injection as the between subjects variable. A significant main effect of injection was observed, $F$ (1,14) = 11.52, $p < 0.05$. Subsequent analysis found that subjects treated with capsaicin showed an increase absorbance at 420 nm (Figure 20A).

The level of hemoglobin-α within the spinal cord tissue was not different between groups. Using quantitative Western blotting, the level of hemoglobin protein at 15 kDa was analyzed with an ANOVA with injection as the between subjects variable. The overall analysis found no significant differences between groups, $F$ (1,14) = 3.79, $p$ = 0.07 (Figure 20B).

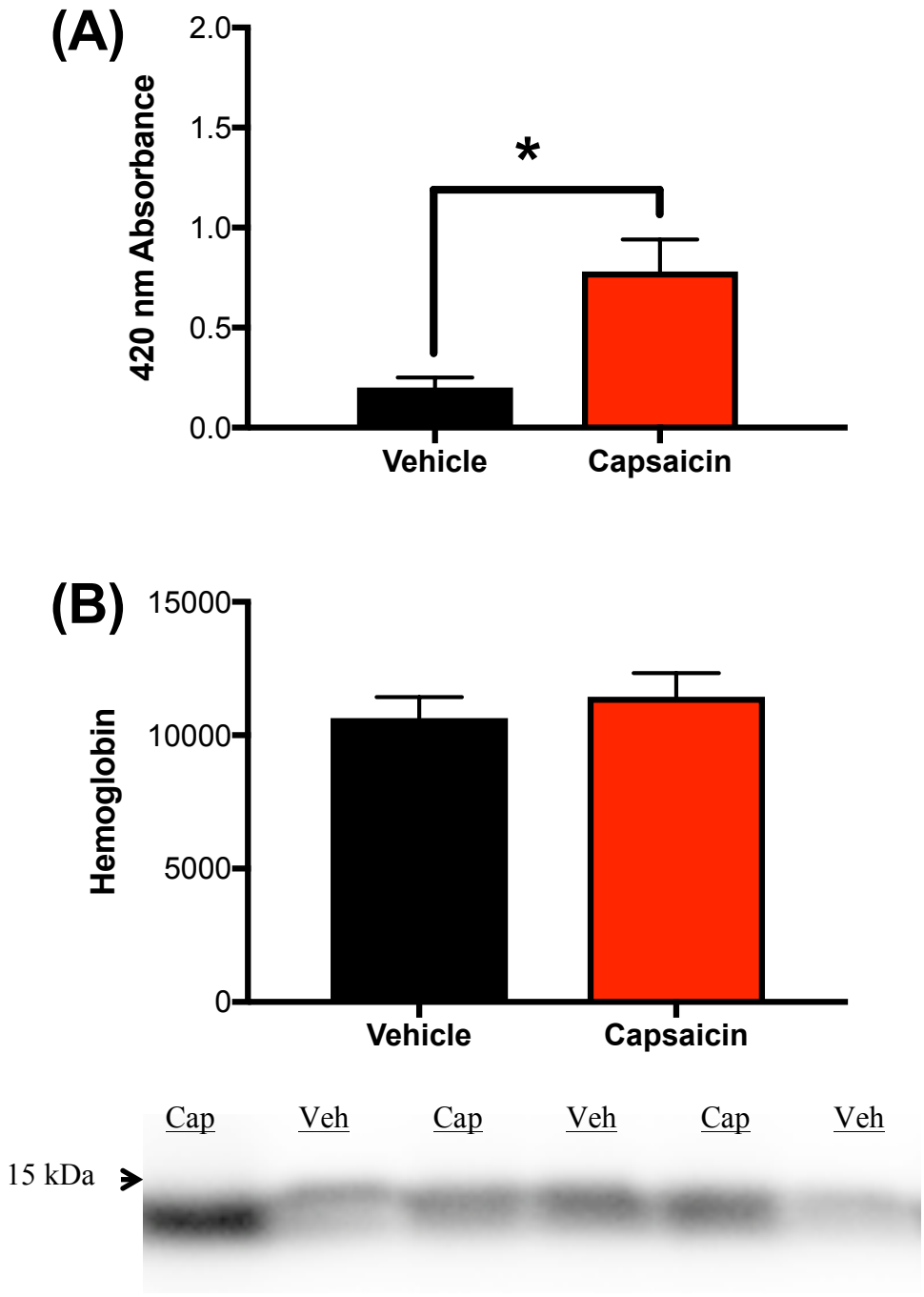


**Figure 20. Hemorrhage 24 hours after capsaicin injection.** The absorbance at 420 nm taken at 24 hours was increased after capsaicin treatment. Error bars represent SEM (n = 8).

Blood pressure measurements did not differ between groups prior to treatment. Pre-treatment values ranged from 96.95 (± 5.78) to 111.68 (± 8.73) mmHg for systolic, 63.66 (± 2.44) to 74.53 (± 5.51) mmHg for diastolic, 74.42 (± 3.27) to 86.60 (± 6.52) mmHg for MAP, 226.53 (± 16.54) to 296.70 (± 37.60) bpm for heart rate, and 2.38 (± 0.64) to 3.74 (± 1.11) μL per minute for blood flow. Independent ANOVAs revealed no difference between groups on any of these measures, $F < 3.25$, $p > 0.05$.

Capsaicin treatment had no effect on hypertension in the first 24 hours after injection. Blood pressure measurements were obtained at three, six, 12, and 24 hours after injection to examine the effect of injection on systolic blood pressure. A repeated measures ANCOVA with injection as the between subjects variable, baseline as the covariate, and time as a repeated measure found no significant changes in systolic blood pressure, $F < 4.259$, $p > 0.05$, although the main effect for systolic blood pressure was marginally significant, $p = 0.06$ (Figure 21A). Neither diastolic blood pressure or MAP showed a significant effect, $F < 2.91$, $p > 0.05$

Capsaicin treatment had no effect on heart rate. Heart rates were obtained at three, six, 12, and 24 hours after injection. A repeated measures ANCOVA with injection as the between subjects variable, baseline as the covariate, and time as a repeated measure, found no significant changes in in heart rate, all $Fs < 1.0$, $p > 0.05$ (Figure 21B). Preplanned analysis of the three hours time point using an ANCOVA revealed no significant increase in heart rate, $F(1,14) < 1.20$, $p > 0.05$.

Capsaicin treatment had no effect on blood flow. Blood flow measurements were obtained at three, six, 12, and 24 hours after injection. A repeated measures ANCOVA

with injection as the between subjects variable, baseline blood flow as a covariate, and time as a repeated measure found no significant changes in blood flow, all $Fs < 2.70$, $p > 0.05$ (Figure 21C). Preplanned analysis of the three hours time point using an ANCOVA revealed no significant increase in blood flow, $F(1, 14) = 3.31$, $p = 0.09$.

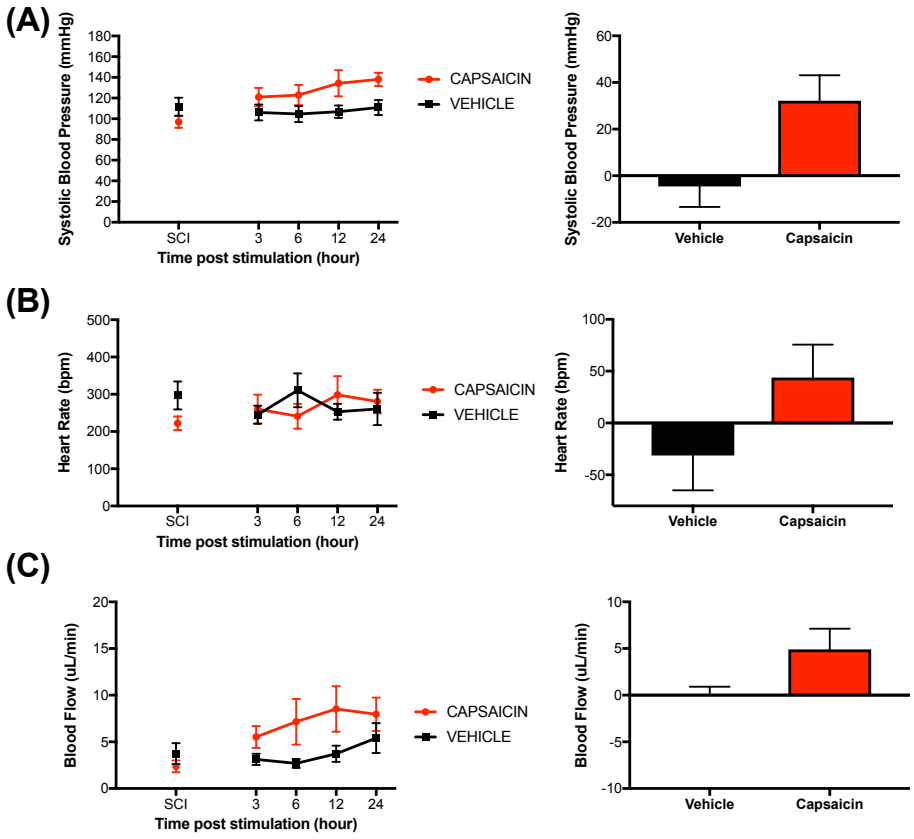


**Figure 21. Blood pressure measurements over the 24 hours after capsaicin injection.** Capsaicin treatment did not increase blood pressure measurements. Error bars represent SEM (n = 8).

Changes in blood pressure measurements (i.e., systolic and diastolic blood pressure, MAP, heart rate and blood flow) were not correlated with hemorrhage at 24 hours in the capsaicin and vehicle group, $r < 0.67$, $p > 0.05$ (Figure 22A-C).

Lower locomotor scores were correlated with an increase in hemorrhage in vehicle treated animals. Using a Pearson's correlation coefficient, I assessed the relationship between locomotor performance and the absorbance of hemoglobin (420 nm) in tissue collect at 24 hours. A strong negative relationship was found between locomotor score at three hours and hemorrhage, $r = -0.79$, $p < 0.05$. Locomotor performance was not correlated with hemorrhage in capsaicin subjects, $r < 0.53$, $p > 0.05$. The scatter plot displayed in Figure 22G shows the relationship between locomotor scores at three hours and hemorrhage at 24 hours.

As blood pressure measurements at multiple time points were highly correlated with locomotor scores at multiple time points, I simplified out analysis by focusing on the three-hour time point for locomotor performance because it was highly correlated with hemorrhage in the above section.

Hypertension was correlated with locomotor performance. Pearson's correlation coefficient, I assessed the relationship between blood pressure and locomotor performance. A strong negative correlation was found between locomotor scores at three hours and systolic blood pressure at three and 12 hours after capsaicin injection, $r = -0.73$ and $-0.76$ (respectively), $p < 0.05$. Similarly, diastolic blood pressure and MAP at 12 hours were also correlated with locomotor scores at three hours, $r = -0.72$, $p < 0.05$. For vehicle controls, locomotor scores at three hours were correlated with diastolic blood

pressure at three hours and MAP at 24 hours, $r$ = -0.81 and 0.76, (respectively), $p < 0.05$. The scatter plot displayed in Figure 22D shows the relationship between systolic blood pressure and locomotor scores at three hours.

Locomotor performance at three hours did not correlated heart rate or blood pressure in capsaicin and vehicle subjects, $r < -0.53$, $p > 0.05$ (Figure 22E-F).

*Discussion*

An increase in hemorrhage was seen 24 hours after capsaicin injection. This replicates a previous study that shows an increase in hemorrhage at three hours that peaks at 24 hours after injection (Turtle et al., 2015) .

No change in blood pressure, heart rate, or blood flow was seen in the current study. This partially replicates the previous study. In that study, heart rate was increased at three hours. This did not replicate in the current study. This suggests that heart rate may not be affected by capsaicin injection. In the previous study, I also saw an increase in blood flow across the first three hours. While only marginally significant in the current study, there was a definite trend for an elevation after capsaicin treatment.

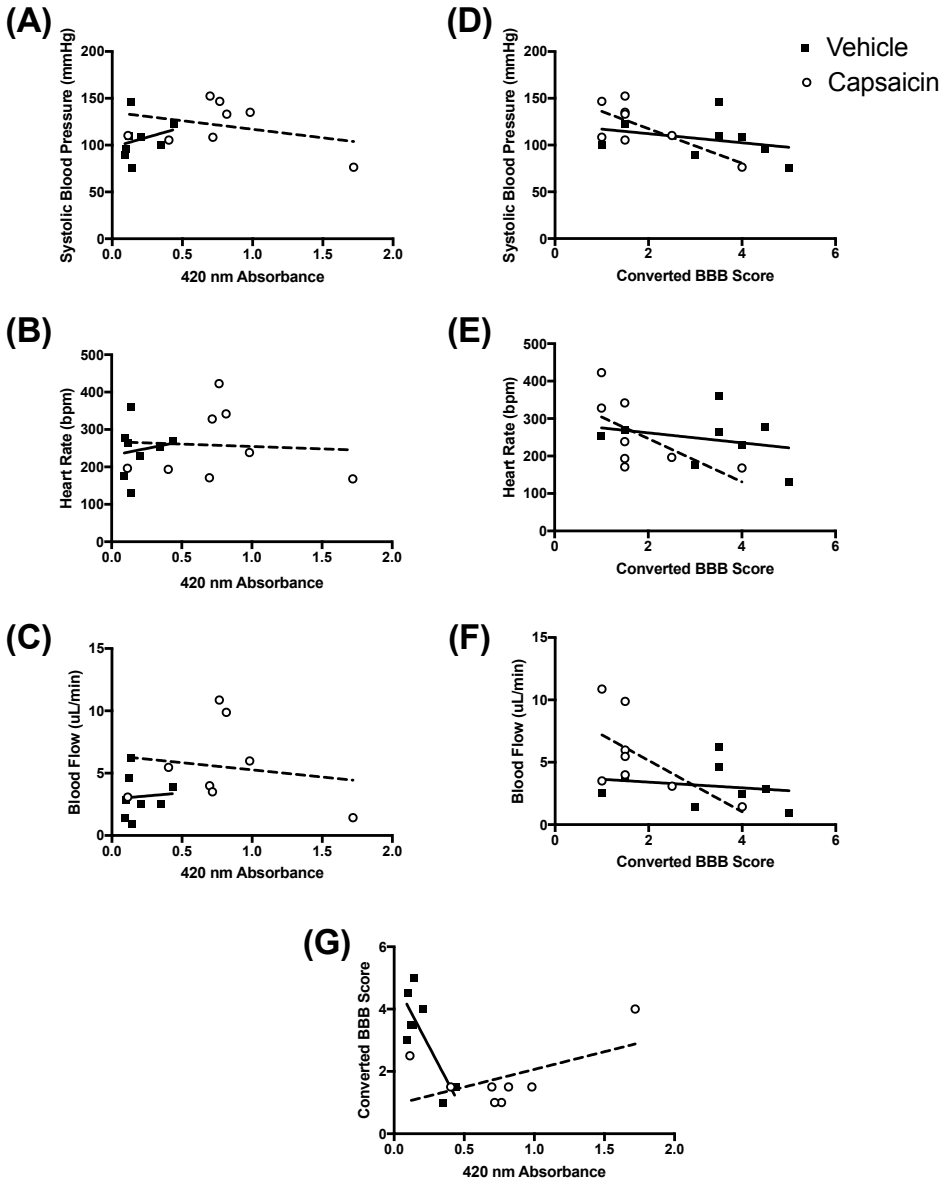


**Figure 22. Scatterplot of groups a day after capsaicin injection.** (A-C) shows a scatter plot of blood pressure measurements at three hours and hemorrhage at 24 hours. (D-F) show a scatter plot of blood pressure measurements and locomotor scores obtained three hours after injection. (G) shows a scatter plot of locomotor scores at three hours and hemorrhage at 24 hours. (n = 8)

# CHAPTER VI

# BEHAVIORAL VARIABLES AFFECTING HEMORRHAGE AND BLOOD PRESSURE

The above studies showed that noxious electrical stimulation after SCI is detrimental to locomotor performance after SCI, collaborating with previous research by Grau and colleagues (Grau et al., 2004). Interestingly, noxious stimulation is given in a controllable manner can inhibit the development of maladaptive plasticity in spinal transected rats (Grau et al., 2004). Additionally, animals given controllable stimulation after a contusion do not show the deficit in recovery seen after intermittent (uncontrollable) stimulation (Grau et al., 2012).

In the current experiment, I examined whether the Master-yoked difference in locomotor performance was related to changes in hemorrhage and blood pressure.

## Experiment 8: Effect of Controllable Shock on Hemorrhage and Blood Pressure

In this experiment I examined whether controllable and uncontrollable shock differentially affects blood pressure measurements, locomotor performance, and hemorrhage expansion.

### *Procedure*

Male Sprague Dawley rats (n = 10) received a moderate spinal cord contusion at T12. The next day, subject had their locomotor scores evaluated using the BBB scale and their baseline blood pressure measurements were taken using a non-invasive blood pressure cuff. Subjects were then set up for instrumental learning and assigned to receive 30 minutes for either Master, Yoked, or unshocked training. Groups were balanced using baseline systolic blood pressure and BBB scores. BBB scores and blood pressure were monitored at one, two and three hours after training. After obtaining the three-hour BBB score and blood pressure measurement, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm).

### *Results*

Subjects that experienced controllable stimulation showed an increase in leg flexion. To examine whether the controllability of shock affected learning, I used a repeated measures ANOVA with training as the between subjects variable and time as the repeated measure. Overall analysis revealed a significant main effect of training and

time, $F > 2.48$, $p < 0.05$ (Figure 23A). No other effects were statistically significant, $F(29, 522) = 1.84$, $p > 0.05$. Follow-up analysis revealed an increase in response duration in Master subjects. Figure 23B shows the number of responses for Master and Yoked subjects. No differences were seen between groups, $F < 1.0$, $p > 0.05$.

BBB scores did not differ between groups prior to treatment. Baseline BBB scores taken 24 hour after spinal cord injury ranged from 4.35 (± 0.55) to 4.80 (± 0.54). An ANOVA revealed no difference between groups, $F(2, 27) < 1.0$, $p > 0.05$.

Short-term locomotor scores were not different between shock groups. BBB scores were obtained one, two, and three hours after stimulation to examine whether the controllability of shock affected locomotor scores. A repeated measures ANCOVA with training as the between subjects variable, baseline BBB scores as the covariate, and time as the repeated measure revealed no significant effects, all $F$s < 1.0, $p > 0.05$ (Figure 24)

Nociceptive stimulation did not induce hemorrhage three hours after shock. The magnitude of the peak in absorbance at 420 nm was analyzed using an ANOVA with training as the between subjects variable. Overall analysis revealed no significant effects, all $F$s < 1.0, $p > 0.05$ (Figure 25).

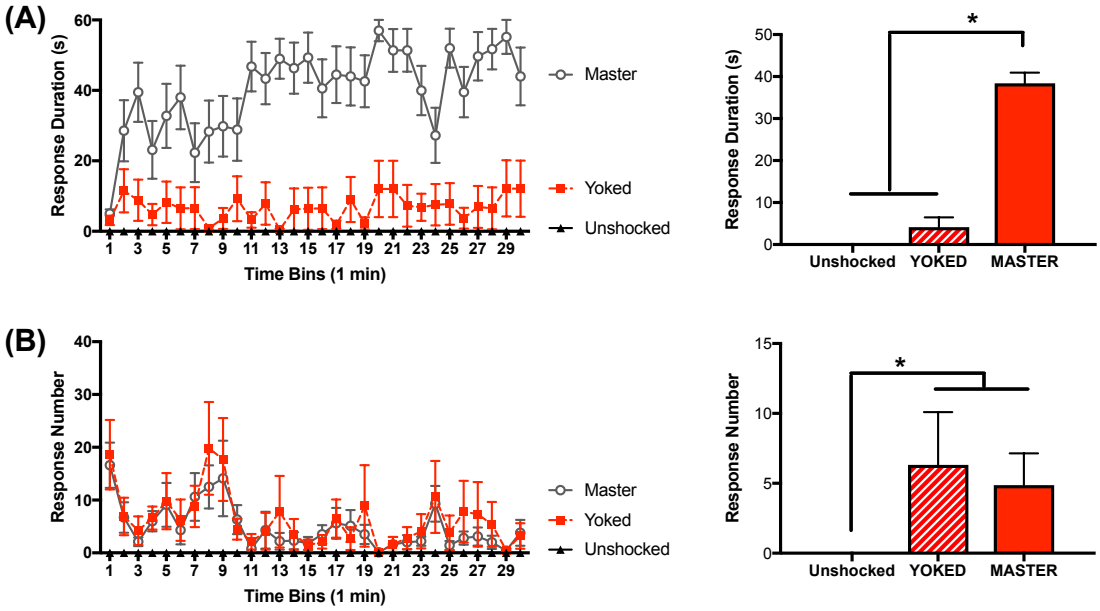


**Figure 23. Instrumental learning after different training schedules.** (A) Subjects receiving controllable stimulation (Masters) exhibited an increase in response duration. (B) Master and Yoked subjects showed a similar number of responses. Error bars represent SEM (n = 10).


**Figure 24. Locomotor performance three hours different training schedules.** Locomotor scores taken during the three hours post stimulation period was not different across the different types of shock. Error bars represent SEM (n = 10).

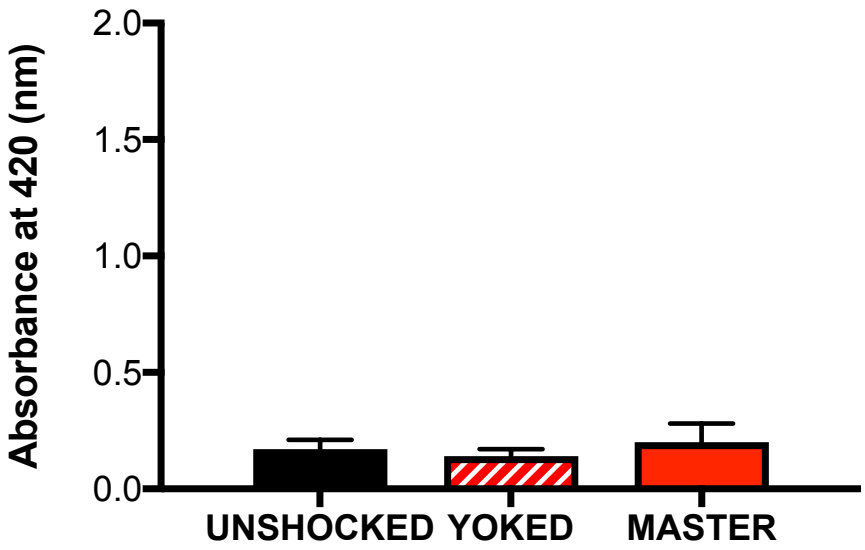


**Figure 25. Hemorrhage three hours after different training schedules.** The absorbance at 420 nm taken at three hours was not different across the different types of shock. Error bars represent SEM (n = 10).

Blood pressure measurements were not different between groups prior to treatment. Pre-treatment values ranged from 109 (± 6.15) to 109.96 (± 7.14) mmHg for systolic, 70.21 (± 3.91) to 71.57 (± 4.88) mmHg for diastolic, 83.16 (± 4.86) to 83.96 (± 5.54) mmHg for MAP, 244.15 (± 25.69) to 274.60 (± 25.94) bpm for heart rate, and 3.52 (± 0.75) to 4.91 (± 1.15) μL per minute for blood flow. Independent ANOVAs revealed no difference between groups on any of these measures, $F$ (2, 27) < 1.0, $p$ > 0.05.

Subject that experienced controllable stimulation had lower blood pressure then subjects experiencing uncontrollable shock. Blood pressure measurements were obtained at one, two and three hours after stimulation to examine whether the controllability of shock affected systolic blood pressure. A repeated measures ANCOVA with training as the between subjects variable, baseline as the covariate, and time as a repeated measure found a main effect of training, $F$ (2, 26) = 3.436, $p$ < 0.05 (Figure 26A). No other effects were statistically significant, $F$ < 1.0, $p$ > 0.05. *Post hoc* analysis of the main effect found a significant difference in hypertension between Master and Yoked subjects. No other groups were statistically different, $p$ > 0.05. Identical results was found for diastolic blood pressure and MAP, $F$ (2, 26) > 3.52, $p$ < 0.05 (data not shown).

Heart rate was not different between shock conditions. Heart rate was obtained at one, two and three hours after stimulation to examine whether the controllability of shock affected heart rate. A repeated measures ANCOVA with training as the between subjects variable, baseline as the covariate, and time as a repeated measure found no significant effects, $F$ > 2.57, $p$ < 0.05 (Figure 26B).

Blood flow was not different between shock conditions. Blood flow was obtained at one, two and three hours after stimulation to examine whether the controllability of shock affected blood flow. A repeated measures ANCOVA with training as the between subjects variable, baseline as the covariate, and time as a repeated measure found no significant effects, $F > 1.0$, $p < 0.05$ (Figure 26C).

*Discussion*

In this study I replicated the increase in flexion duration seen after controllable stimulation in contused subject. I did not see a change in locomotor performance over the three-hour post stimulation period. As mentioned above, the deficit in locomotor performance may take longer to develop. In previous work, for example, exposure to Yoked stimulation causes a long-term decrease in locomotor recovery that emerged after the first week (Grau et al., 2004).

Unexpectedly, I did not see an increase in hemorrhage expansion after shock exposure. This could be due to differences in shock between this study and the previous studies. First, many subjects received significantly fewer shocks then are normally given (ranged from 60 to 307 shocks). Further in this study subjects receive shock to their tibialis anterior muscle instead of the leg. The intensity of the shock was also different (0.6 mA verses 1.5 mA). Additionally, the time-course of shock was different. For tail shock, subjects were exposed to 180 shocks within six minutes. For learning, subjects were exposed to an average of 146 shocks over 30 minutes. These differences in parameters may play a role in changing the time course of hemorrhage or amount of hemorrhage observed.

Interestingly, I did see a difference in hypertension between Master and Yoked subjects, with Master subjects decreasing their blood pressure and Yoked subjects increasing their blood pressure. This was interesting because the amount of shock was identical between these groups. This suggests that controllability of stimulation affects the cardiovascular system after SCI.

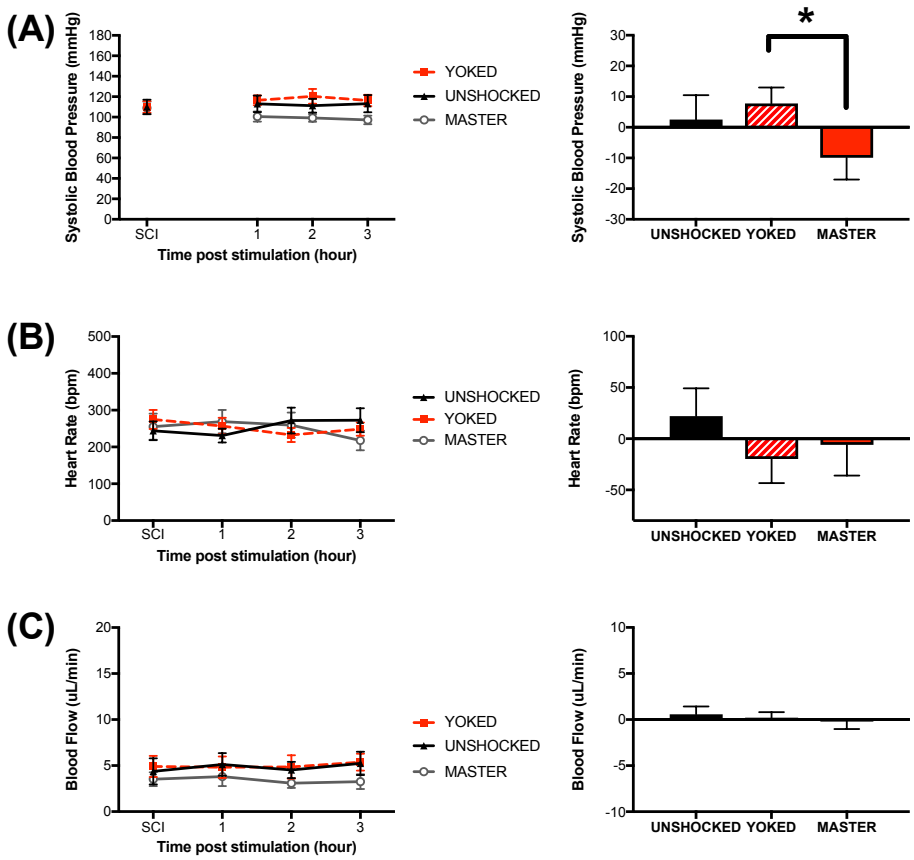


**Figure 26. Blood pressure measurements three hours after different training schedules.** Master and yoked subjects differed on systolic blood pressure across the three hours post shock period. Error bars represent SEM (n = 10).

# CHAPTER VII

# EFFECT OF SEX ON HEMORRHAGE AND LOCOMOTION AFTER NOXIOUS INPUT

The incidence of spinal cord injuries in females has been increasing in the last few years (DeVivo & Chen, 2011). Yet there has been little research examining how pain input affects females after SCI. Studies examining sex differences have observed that females show better outcomes than males after SCI and other central nervous system injuries (Datto, Yang, et al., 2015). While Grau and colleagues (2004) have shown that nociceptive input (i.e., shock and capsaicin) was detrimental to recovery after spinal cord injury in males, it is not know whether these same effects would be observed in females.

In the current set of experiments, I aimed to determine whether pain input causes a change in hemorrhage, blood pressure and locomotor performance in females rats. Experiment 9 exposed female rats to uncontrollable electrical stimulation whereas Experiment 10 exposed females to capsaicin. Females were compared to age and weight match males in both experiments.

## Experiment 9: Effect of Shock on Blood Pressure and Hemorrhage in Females

In this experiment, I examined 12-week-old females, 12-week-old males and 7-week-old males to see if they differed on shock-induced hemorrhage expansion, locomotor behavior, and blood pressure.

### *Procedure*

Twelve-week-old Sprague Dawley female rats (227.93 ± 2.13 grams) were compared with male Sprague Dawley rats aged seven weeks (232.69 ± 4.23 grams) and 12 weeks (321.10 ± 2.71 grams). Rats (n = 8) received a moderate spinal cord contusion at T12. The next day subjects had their locomotor scores evaluated using the BBB locomotor scale and their baseline blood pressure measurements taken using a non-invasive blood pressure cuff. Subjects were then randomly assigned to receive six minutes of electrical stimulation (180 shocks, 1.5mA, 0.2-3.8 second ISI) to the tail or remained unshocked. Groups were balanced using baseline systolic blood pressure and BBB scores. BBB scores and blood pressure measurements were monitored at one, two and three hours after electrical stimulation. After obtaining the three hours BBB score and blood pressure measurement, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm).

*Results*

Locomotor scores did not differ between groups prior to treatment. BBB scores obtained the day after surgery, prior to treatment, ranged from 4.19 (± 0.53) to 4.69 (± 0.57). An ANOVA revealed no difference between groups, $F(1, 28) < 1.0$, $p > 0.05$.

Electrical stimulation reduced locomotor scores regardless of sex. BBB scores were obtained at one, two, and three hours after stimulation to examine whether sex affected locomotor scores. A repeated measures ANCOVA with shock and sex as the between subjects variable, baseline BBB scores as the covariate, and time as the repeated measure revealed a main effect of shock, $F(1, 41) = 7.40$, $p < 0.05$. No other effects were statistically significant, $F(1, 27) < 1.0$, $p > 0.05$ (Figure 27). Analysis of the main effect showed that electrical stimulation produced lower BBB scores compared to unshocked controls.

Females showed more overall hemorrhage at three hours compared to weight-matched controls. Additionally, hemorrhage at three hours was increased in subjects that received electrical stimulation. The magnitude of the peak in absorbance at 420 nm was analyzed using an ANOVA with sex and shock as the between subjects variable. The overall analysis revealed a main effect of sex and shock, $F(2, 47) > 5.00$, $p < 0.05$ (Figure 28). No other effects were statistically significant, $F(1, 27) < 1.18$, $p > 0.05$. *Post hoc* comparison of the main effect of shock found that animals exposed to electrical stimulation showed more hemorrhage. Analysis of the main effect of sex found that the amount of hemorrhage in females was increased compared to seven-week-old males.

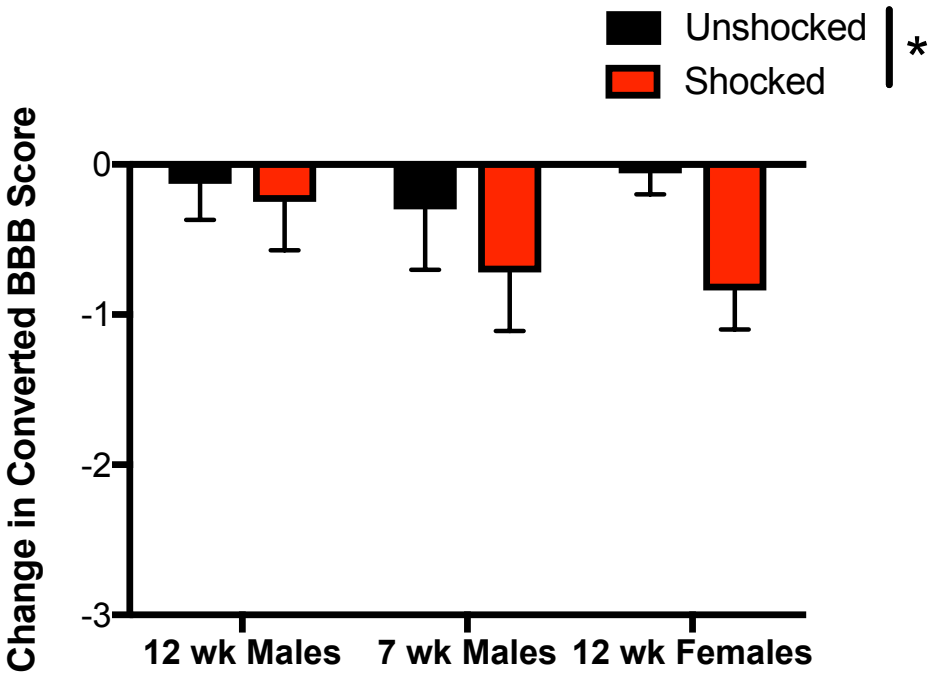


**Figure 27. Locomotor performance over the three hours after shock in males and females.** Locomotor scores over the three hours post stimulation period significantly decreased with stimulation. Error bars represent SEM (n = 8).

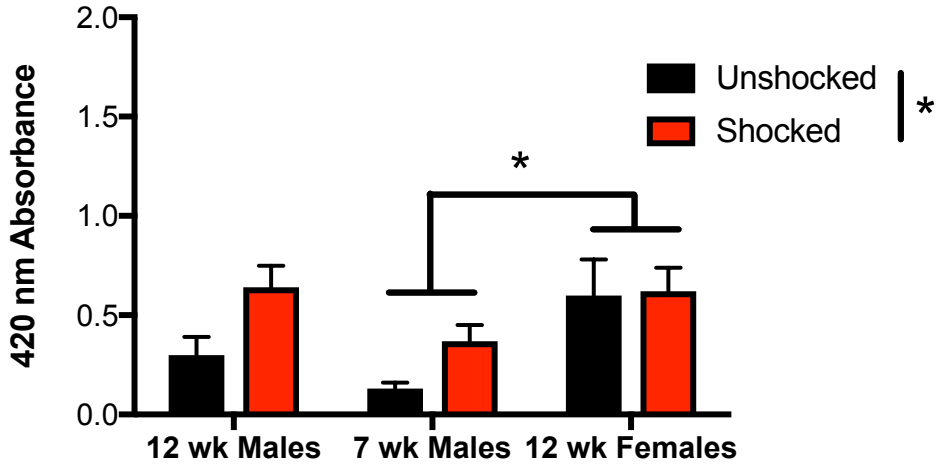


**Figure 28. Hemorrhage three hours after shock in males and females.** The absorbance at 420 nm taken at three hours was increased in animals receiving noxious stimulation. Females showed more hemorrhage then seven-week-old males. Error bars represent SEM (n = 8).

Blood pressure measurements were different between groups prior to treatment. Pre-treatment values ranged from 102.85 (± 8.74) to 120.32 (± 4.79) mmHg for systolic, 66.08 (± 6.38) to 81.85 (± 4.66) mmHg for diastolic, 78.46 (± 7.64) to 94.56 (± 3.46) mmHg for MAP, 234.15 (± 33.22) to 352.99 (± 42.55) bpm for heart rate, and 3.32 (± 0.99) to 8.23 (± 1.61) μL per minute for blood flow. Independent ANOVAs, with sex and shock as between subjects variable, revealed a main effect of sex for diastolic blood pressure, MAP, and blood flow, all $F$s < 3.38, $p > 0.05$. *Post hoc* comparison of the group means showed that the 12-week-old males had lower values compared to females and seven week old males.

Electrical stimulation increased systolic blood pressure. Systolic blood pressure was obtained immediately, one, two and three hours after stimulation to examine how sex and shock affected the development of hypertension. A repeated measures ANCOVA with sex and shock as the between subjects variable, baseline at the covariate, and time as the repeated measure, found a main effect of shock, $F$ (1, 41) = 5.00, $p <$ 0.05 ( Figure 29A). No other effects were statistically significant, $F < 1.91$, $p > 0.05$. *Post hoc* comparison of the main effect revealed an increase in systolic blood pressure after electrical stimulation. There were no significant effects for diastolic blood pressure and MAP, $F < 3.68$, $p > 0.05$ (data not shown).

Females had higher heart rates compared to age-matched males. In addition, electrical stimulation increased heart rate soon after stimulation. One subject in the unshocked female group was excluded from analysis due to lack of heart rate data. A repeated measures ANCOVA with sex and shock as the between subjects variable,

baseline heart rate at the covariate, and time as the repeated measure revealed a main effect of sex and an interaction between time by shock, $F > 2.6$, $p < 0.05$ ( Figure 29B). No other effects were statistically different, $F < 2.04$, $p < 0.05$. *Post hoc* analysis of the main effect of sex found a significant different between females and 12 week old males. The difference between females and seven week old males was marginally significant, $p = 0.07$. No other groups were statistically significant, $p < 0.05$. Analysis of the interaction between time and shock found an increase in heart rate an hour after shock exposure.

Blood flow was increased in subjects after electrical stimulation. To examine how sex and shock affected blood flow, I used a repeated measures ANCOVA with sex and shock as the between subjects variable, baseline at the covariate, and time as the repeated measure. The omnibus analysis found a main effect of time and shock and an interaction between time and sex, $F > 4.18$, $p < 0.05$. No other effects were statistically significant, $F < 2.77$ $p > 0.05$. *Post hoc* comparison of the main effect of shock showed an increased blood flow after electrical stimulation ( Figure 29C). Analysis of the interaction between time and sex found a significant decrease in blood flow in seven-week-old males two hours after stimulation.

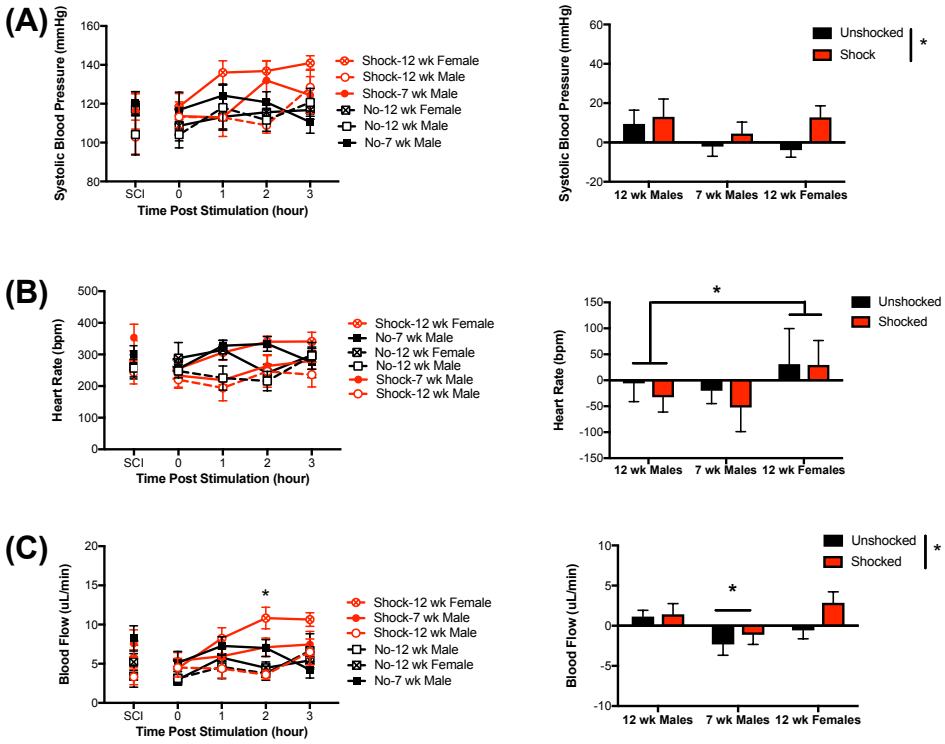


**Figure 29. Blood pressure three hours after shock in males and females.** Blood pressure, heart rate, and blood flow were increase in subjects receiving electrical stimulation. Females tended to have higher heart rates. Twelve-week-old animals had higher blood flow. Error bars represent SEM (n = 8).

Hemorrhage was not correlated with hypertension for any group, $r < 0.68$, $p > 0.05$ (data not shown).

Elevated heart rate was correlated with hemorrhage in 12-week-old males receiving shock. Using a Pearson's correlation coefficient, I assessed the relationship between heart rate and absorbance of hemoglobin (420 nm) in tissue collected at 3 hours. A strong positive correlation was found between heart rate at three hours and hemorrhage 12-week-old males that received electrical stimulation, $r = 0.84$, $p < 0.01$ (data not shown).

An increase in blood flow was correlated with increase in hemorrhage in unshocked seven-week-old males. Using a Pearson's correlation coefficient, I assessed the relationship between blood flow and absorbance of hemoglobin (420 nm) in tissue collected at three hours. A strong positive correlation was found between blood flow at three hours and hemorrhage unshocked seven-week-old males, $r = 0.78$, $p < 0.05$(data not shown).

A decrease in locomotor scores was correlated with an increase in hemorrhage in females that received shock and unshocked 12-week-old males. Using a Pearson's correlation coefficient, I assessed the relationship between locomotor scores and absorbance of hemoglobin (420 nm) in tissue collected at three hours. A strong negative was found between BBB scores taken immediately, two, and three hours after shock in shocked females ($r$ = -0.76, -0.98, and -0.74) and unshocked 12-week-old males ($r$ = -0.92, -0.83, and -0.83), $p < 0.05$. There was also a negative correlation between BBB

scores at 1 hour and hemorrhage in unshocked 12 week old males, $r = -0.87$, $p < 0.05$ (data not shown).

As blood pressure measurements at multiple time points were highly correlated with locomotor scores at multiple time points, I simplified the analysis by focusing on the three-hour time point for locomotor performance because it was highly correlated with hemorrhage in the above section.

Hypertension was correlated with locomotor scores in unshocked males and shocked females. Using a Pearson's correlation coefficient, I assessed the relationship between blood pressure and locomotor scores. A strong negative correlation was found between systolic blood pressure, diastolic blood pressure, and MAP at 3 hours and locomotor score at 3 hours for unshocked seven week old males, $r = -0.74$, -0.83, and -0.81 (respectively), $p < 0.05$. A strong negative correlation was found between diastolic blood pressure and MAP at two hours and locomotor scores at three hours in shocked females, $r = -0.77$ and -0.74 (respectively), $p < 0.05$. A strong positive correlation was found between diastolic blood pressure and MAP at three hours and locomotor scores at three hours in unshocked 12 weeks old males, $r = 0.86$ and 0.82 (respectively), $p < 0.05$ (data not shown).

An elevation in heart rate was correlated with an increase in locomotor scores in unshocked 12-week-old males. Using a Pearson's correlation coefficient, I assessed the relationship between heart rate and locomotor scores. A strong positive correlation was found between heart rate at one, two and three hours and locomotor scores at three hours

for unshocked 12-week-old males, $r = 0.87$, 0.74, and 0.72 (respectively), $p < 0.05$ (data not shown).

An increase in blood flow was correlated to a decrease in locomotor scores in females and unshocked seven-week-old males. Using a Pearson's correlation coefficient, I assessed the relationship between blood flow and locomotor scores. A strong negative correlation was found between immediate blood flow in unshocked males, between blood flow at two hours in shocked females, and between blood flow at three hours in unshocked females with locomotor scores, $r = -0.73$, -0.80, and -0.78, (respectively), $p < 0.05$ (data not shown).

*Discussion*

Simulation caused a decreased in locomotor performance and an increase in hypertension and blood flow. This replicated our previous results and suggests that females are not protected from the detrimental effect of shock on locomotor performance and blood pressure. It should be noted that the effect of stimulation on locomotor performance and blood pressure in males was not as strong as in Experiment 4. It will be important in future studies to isolate the factors that influence the effect of nociceptive stimulation on recovery.

Stimulation caused an increase in hemorrhage in male rats. This replicated previous work and confirms our previous result (Turtle et al., 2017). However, females did not show an increased hemorrhage in response to stimulation. Further, the amount of hemorrhage seen in the female group was higher then the male groups. Work would

should examine whether this hemorrhage seen in females results in a deficit in long-term recovery.

Females showed an elevation in heart rate compared to age matched males at baseline and throughout the study. Given we didn't see a significant difference between weight match controls, this elevation in heart rate might be explained by differences in the size of the hearts between age matched males and females. Research has shown that females tend to have more rapid heart rates then males due in part to smaller hearts a vessels, which have to pump faster to maintain similar outputs (Glinskii et al., 2007).

## Experiment 10: Effect of Capsaicin on Blood Pressure and Hemorrhage in Females

Experiment 9 showed that intermittent shock impaired locomotor performance and induced an increase in blood pressure in female rate, but did not produce greater hemorrhage. In this experiment, I examined whether capsaicin caused a similar effect in males and females.

### *Procedure*

Twelve-week-old Sprague Dawley female rats (241.07 ± 2.75 grams) were compared with male Sprague Dawley rats aged seven weeks (232.43 ± 2.57 grams) and 12 weeks (321.43 ± 2.46 grams). Rats (n = 7) received a moderate spinal cord contusion at T12. The next day subjects had their locomotor scores evaluated using the BBB locomotor scale and their baseline blood pressure measurements taken using a non-invasive blood pressure cuff. Subjects were then randomly assigned to receive an intradermal injection of capsaicin (3%) or vehicle. Groups were balanced using baseline systolic blood pressure and BBB scores. BBB scores and blood pressure were then monitored beginning immediately, one, two, and three hours after injection. After obtaining the three hours BBB score and blood pressure measurement, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm).

*Results*

BBB score did not differ between groups prior to treatment. Baseline BBB scores taken three hour after spinal cord injury ranged from 5.07 (± 0.38) to 6.44 (± 0.70). An ANOVA revealed no differences between groups, $F < 1.33$, $p > 0.05$.

Locomotor scores were not different within three hours after electrical shock for any group. BBB scores were obtained one, two, and three hours after stimulation to examine whether sex and injection affected locomotor scores. A repeated measures ANCOVA with sex and injection as the between subjects variable, baseline BBB scores as the covariate, and time as the repeated measure revealed no significant effects, $F > 3.93$, $p < 0.05$ (Figure 30).

Capsaicin treatment did not change the extent of hemorrhage after spinal cord injury in any of the groups. The magnitude of the peak in absorbance at 420 nm was analyzed using an ANOVA with injection and group as the between subjects variable. No significant effects were observed, $F < 3.04$, $p > 0.05$ (Figure 31).

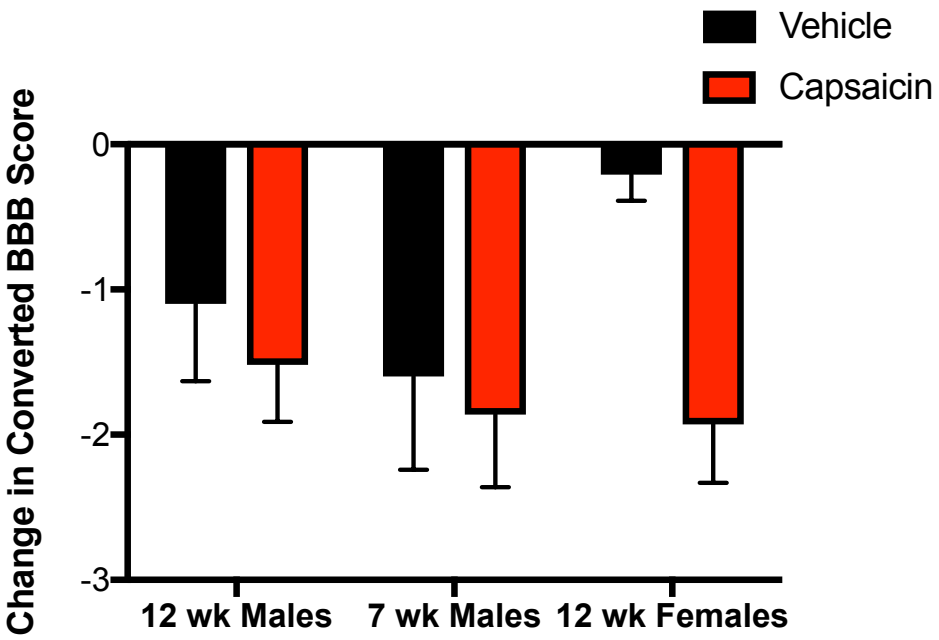


**Figure 30. Locomotor performance over the three hours after capsaicin injection in males and females.** There was no change in locomotor scores after capsaicin treatment in males and females. Error bars represent SEM (n = 7).

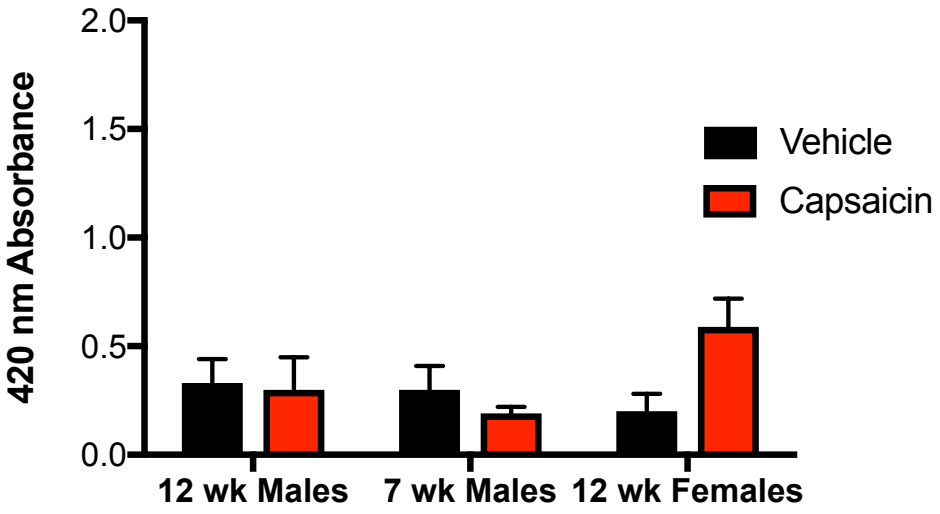


**Figure 31. Hemorrhage three hours after capsaicin treatment in males and females.** The absorbance at 420 nm was not difference after capsaicin treatment in males and females after three hours. Error bars represent SEM (n = 7).

Blood pressure measurements differed between groups prior to treatment. Pre-treatment values ranged from 101.02 (± 6.30) to 122.47 (± 6.98) mmHg for systolic, 67.79 (± 2.84) to 86.63 (± 8.97) mmHg for diastolic, 78.51 (± 3.8) to 96.84 (± 9.55) mmHg for MAP, 215.87 (± 45.46) to 362.05 (± 31.29) bpm for heart rate, and 3.65 (± 1.02) to 11.20 (± 2.08) μL per minute for blood flow. Independent ANOVAs revealed a main effect of sex for systolic, diastolic, MAP, heart rate and blood flow, all $F$s > 3.44, $p < 0.05$. *Post hoc* comparisons revealed decreased baseline measures in females compared to the males.

Lower systolic blood pressure was seen in female subjects. Systolic blood pressure was obtained immediately, one, two and three hours after injection to examine how sex and injection affected systolic blood pressure. A repeated measures ANCOVA with sex and injection as the between subjects variable, baseline as the covariate, and time as the repeated measure found a main effect of time and sex, $F > 3.86$, $p < 0.05$ (Figure 32A). No other effects were statistically significant, $F < 1.33$, $p > 0.05$. *Post hoc* comparison of the main effect of sex found that females had lower systolic blood pressure compared to seven-week-old males. A marginally significant effect between females and 12 week old males was also seen, $p = 0.06$. The main effect of time indicated a general decrease in systolic blood pressure over time. Identical results were seen for diastolic blood pressure and MAP, $F > 3.23$, $p < 0.05$ (data not shown).

Heart rate was not different across groups. To examine how sex and injection affected heart rate, I used a repeated measures ANCOVA with sex and injection as the

between subjects variable, baseline as a covariate, and time as the repeated measure. The omnibus analysis found no significant effects, $F > 1.48$, $p < 0.05$.

Blood flow increased over time in all groups. To examine how sex and injection affected blood flow, I used a repeated measures ANCOVA with sex and injection as the between subjects variable, baseline as a covariate, and time as the repeated measure. The omnibus analysis found a main effect of sex and time, $F > 3.54$, $p < 0.05$ Figure 32B. No other effects were statistically significant, $F < 2.0$, $p > 0.05$. *Post hoc* comparison of the main effect of sex found that seven-week-old males had significantly higher blood flow. The main effect of time shows a general decrease in blood flow over time.

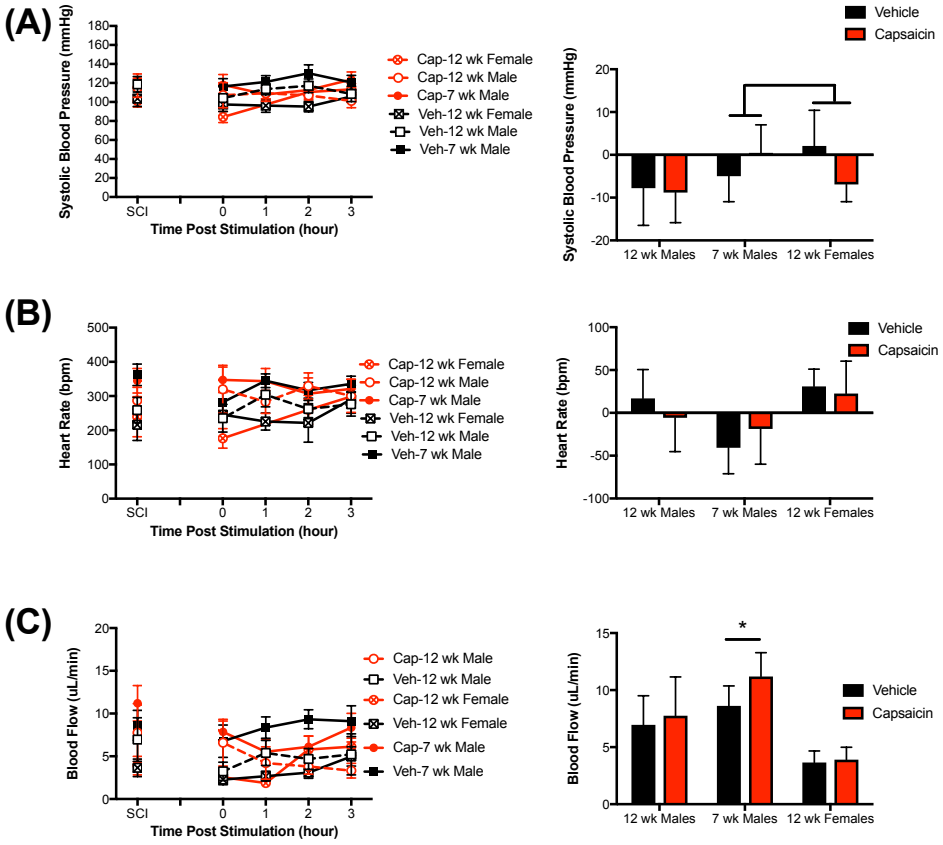


**Figure 32. Blood pressure over the three hours after capsaicin injection in males and females.** (A) Females had lower systolic blood pressure compared to males. (B) Heart rate was not difference between groups. (C) Seven-week-old males had higher blood flow compared to females and 12-week-old males. Error bars represent SEM (n = 7).

Blood pressure measurements (systolic and diastolic blood pressure, MAP, heart rate, and blood flow) were not correlated with hemorrhage in any group, $r < 0.72$, $p > 0.05$. Heart rate was not analyzed due to a large number of missing values in the female groups (data not shown).

Locomotor behavior was not correlated with hemorrhage in any group, $r < 0.74$, $p > 0.05$ (data not shown).

Hypertension was correlated with an increase in locomotor scores in capsaicin females and vehicle males. Using a Pearson's coefficient, I assessed the relationship between blood pressure and locomotor scores. In capsaicin females, a strong positive correlation was found between immediate changes in systolic and diastolic blood pressure and MAP and locomotor scores at one hour, $r = 0.77, 0.79, 0.79$. Additionally, capsaicin females had a correlation between systolic and diastolic blood pressure and MAP at three hours and locomotor scores at two ($r = 0.88, 0.81$, and 0.86) and three hours ($r = 0.86, 0.91$, and 0.92). In vehicle seven week males, systolic, diastolic blood pressure and MAP at one hour was correlated with locomotor scores at one ($r = 0.82$, 0.85, and 0.86) and two hours ($r = 0.82, 0.84$, and 0.85). Finally, in vehicle 12-week-old males systolic blood pressure at one hour was correlated with locomotor scores at three hours, $r = 0.79$. Hypertension was not correlated with locomotor scores in vehicle females and capsaicin males, $r < 0.66$, $p > 0.05$ (data not shown).

Heart rate was not correlated with locomotor behavior in any of the groups, $r < 0.75$, $p > 0.05$ (data not shown).

Blood flow was not correlated with locomotor behavior in any of the groups, $r < 0.75$, $p > 0.05$ (data not shown).

*Discussion*

In this study, I found no change in locomotor performance and hemorrhage after animals were exposed to capsaicin. This is contrary to the results in Experiment 6, where males showed a robust increase in hemorrhage and a decrease in locomotor performance. The results in the current study seem to be due to an increase in variability in male vehicle subjects as examination of just the females show that capsaicin did cause an increase in hemorrhage and a decrease in locomotor performance. This is actually consistent with previous observations that showed that an injection per se causes an adverse effect in locomotor recovery (Turtle et al., 2015).

Capsaicin did not change blood pressure measurement in both males and females. This replicated our previous findings.

# CHAPTER VIII

# EFFECT OF MANIPULATING BLOOD PRESSURE ON HEMORRHAGE AND LOCOMOTOR PERFORMANCE

In the previous experiments nociceptive stimulation was shown to induce an increase in blood pressure and heart rate. This increase was shown to last at least three hours and correlated with an increase in hemorrhage and a decrease in locomotor performance. Similarly, others have found that changes in blood pressure around the after injury correlated with long-term locomotor recovery outcomes (Nielson et al., 2015).

To examine whether blood pressure is necessary and sufficient for the development of hemorrhage, I pharmacologically lowered and raised the blood pressure after SCI. In Experiment 11, prazosin was used to block the rise in blood pressure produced by nociceptive stimulation. Prazosin is an alpha-1-adrenoceptor antagonist that has been used to block episodes of autonomic dysreflexia in people with SCI. In Experiment 12, I examined whether prazosin could prevent the long-term effects of electrical stimulation. In Experiment 13, I examined whether multiple doses of prazosin could prevent hemorrhage 24 hours after electrical stimulation. Finally, in Experiment 14, I used norepinephrine to experimentally increase blood pressure.

## Experiment 11: Blocking the Blood Pressure Increase on Hemorrhage at Three Hours

Experiment 11 used prazosin to block the acute rise in blood pressure induced by shock exposure.

### *Procedure*

Twenty-four hours after receiving a moderate spinal cord contusion at T12, male Sprague Dawley rats (n = 8) had their locomotor scores evaluated using the BBB locomotor scale and their baseline blood pressure measurements taken using a non-invasive blood pressure cuff. Subjects were then randomly assigned to prazosin (3 mg/kg) or vehicle (30% glucose). Groups were balanced using baseline systolic blood pressure and BBB scores. Six minutes after the injection of drug, subjects had their blood pressure monitored. Thirty minutes after drug administration, subjects were randomly assigned to electrical stimulation (180 shocks, 1.5mA, 0.2-3.8 second ISI) to the tail or remained unshocked. BBB scores and blood pressure were monitored immediately, one, two, and three hours after electrical stimulation. After obtaining the three hours BBB score and blood pressure measurement, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm).

*Results*

Locomotor scores did not differ between groups prior to drug treatment. BBB scores obtained the day after surgery, prior to treatment, ranged from 3.06 (± 0.37) to 3.5 (± 0.33). An ANOVA revealed no significant difference between groups, $F < 1.0$, $p > 0.05$.

Prazosin did not block the decease in locomotor behavior seen after nociceptive stimulation. To examine whether drug and shock would affect locomotor scores, BBB scores were obtained one, two, and three hours after stimulation. A repeated measures ANCOVA with shock and drug as the between subjects variable, baseline BBB scores as the covariate, and time as the repeated measure revealed a main effect of shock, $F$ (1, 27) = 20.85, $p < 0.001$. No other groups were statistically significant, all $F$s < 1.0, $p > 0.05$. *Post hoc* comparison of the main effect of shock showed decreased locomotor scores in subjects receiving electrical stimulation (Figure 33).

Prazosin blocked the expansion of the hemorrhage. The magnitude of the peak in absorbance at 420 nm was analyzed using an ANOVA with shock and drug as the between subjects variable. Overall analysis revealed a main effect of drug, $F$ (1, 28) = 7.57, $p < 0.01$. No other effects were statistically significant, $F < 3.24$, $p > 0.05$. *Post hoc* comparison of the main effect found that subjects receiving prazosin had reduced indices of hemorrhage, compared to vehicle controls (Figure 34).

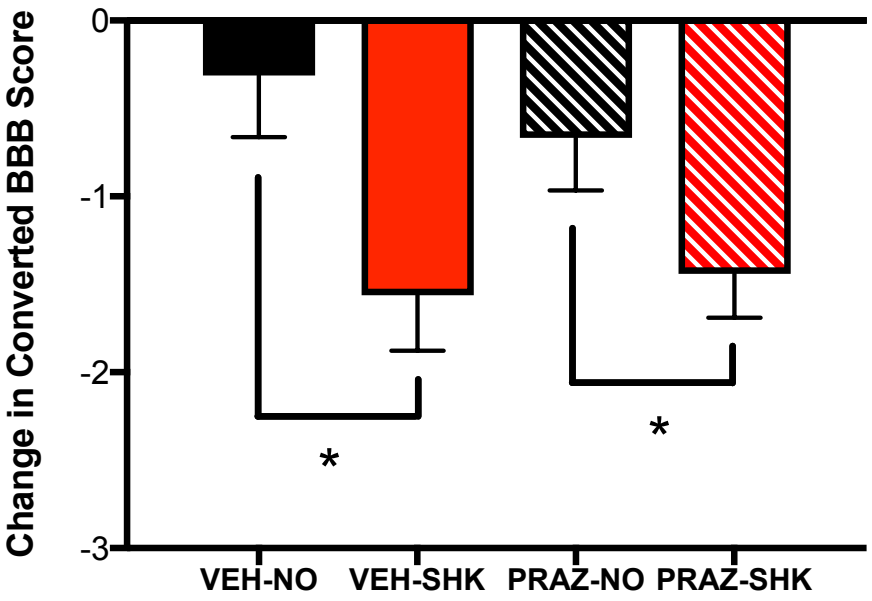


**Figure 33. Locomotor performance over the three hours after prazosin and shock.** Electrical stimulation decreased locomotor performance. Prazosin did not block this effect. Error bars represent SEM (n = 8).

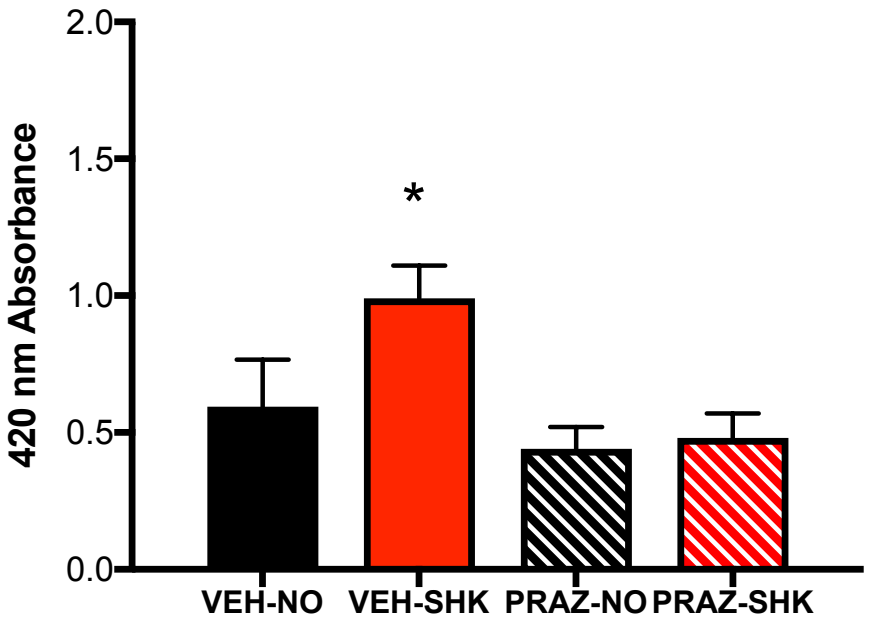


**Figure 34. Hemorrhage three hours after prazosin and shock.** The absorbance at 420 nm taken was increased in vehicle subjects exposed to shock. Prazosin blocked this increase in hemorrhage. Error bars represent SEM (n = 8).

Blood pressure measurements were not different between groups prior to drug. Pre-treatment values ranged from 105.89 (± 4.85) to 109.76 (± 9.63) mmHg for systolic, 69.98 (±) to 73.14 (±) mmHg for diastolic, 82.83 (± 4.30) to 84.97 (± 7.19) mmHg for MAP, 230.51 (± 39.04) to 254.05 (± 18.66) bpm for heart rate, and 3.69 (± 0.71) to 5.18 (± 1.67) μL per minute for blood flow. Independent ANOVA revealed no difference between groups, all $F$s < 1.28, $p > 0.05$. An injection of prazosin significantly lowered heart rate and blood flow, $F$ (1, 32) > 7.20, $p < 0.05$.

Electrical stimulation increased systolic blood pressure in vehicle subjects, but not prazosin subjects. Prazosin increased systolic blood pressure in unshocked controls. Blood pressure measurements were obtained one, two, and three hours after stimulation to examine the effect of stimulation on systolic blood pressure. A repeated measures ANCOVA with stimulation as the between subjects variable, baselines as a covariate, and time as a repeated measure found an interaction between shock and drug, $F > 11.54$, $p < 0.05$ (Figure 35A). No other effects were statistically significant, $F < 2.42$, $p > 0.05$. Analysis of the interaction found an increase in systolic blood pressure in vehicle-shocked subjects, but not in prazosin-shocked subjects. Additionally in unshocked subjects, prazosin alone increased systolic blood pressure. No other groups were significantly different, $F < 1.0$, $p > 0.05$.

Prazosin did not prevent a rise in diastolic pressure and MAP, instead it caused an increase in blood pressure when given to unshocked subjects. Omnibus analysis of diastolic blood pressure and MAP found an interaction between condition and drug, and an three-way interaction between shock, drug, and time, $F > 4.76$, $p < 0.05$ (Figure 35B).

No other effects were statistically significant, $F < 3.70$, $p > 0.05$. *Post hoc* comparison of the interactions showed an increase in diastolic blood pressure and MAP in prazosin treated subjects and vehicle shocked subjects beginning one hour after stimulation compared vehicle unshocked controls.

Prazosin treatment elevated heart rate after SCI. Heart rates were obtained one, two, and three hours after stimulation. A repeated measures ANCOVA with stimulation as the between subjects variable, baseline heart rate as a covariate, and time as a repeated measure found a main effect of drug and an interaction between time by shock, $F > 2.45$, $p < 0.01$. No other effects were statistically significant, $F < 3.43$, $p > 0.05$ (Figure 35C). *Post hoc* comparison of the main effect found that prazosin increased heart rates compared to vehicle controls. Analysis of the interaction between time and shock found that stimulation caused a general increase in heart rate over time.

Prazosin increased blood flow equally in both shocked and unshocked animals. Shock increased blood flow in vehicle treated subjects. Blood flow measurements were obtained immediately, one, two, and three hours after stimulation. A repeated measures ANCOVA with stimulation as the between subjects variable, baseline blood flow as a covariate, and time as a repeated measure found a main effect of drug and condition, and an interaction between drug and condition, $F > 6.58$, $p < 0.05$ (Figure 35D). No other effects were statistically significant, $F < 2.61$, $p > 0.05$. *Post hoc* comparison of the interaction between drug and condition found that prazosin significantly elevated blood flow above vehicle subjects in the shocked and unshocked groups. Within the vehicle group, shocked increased blood flow.

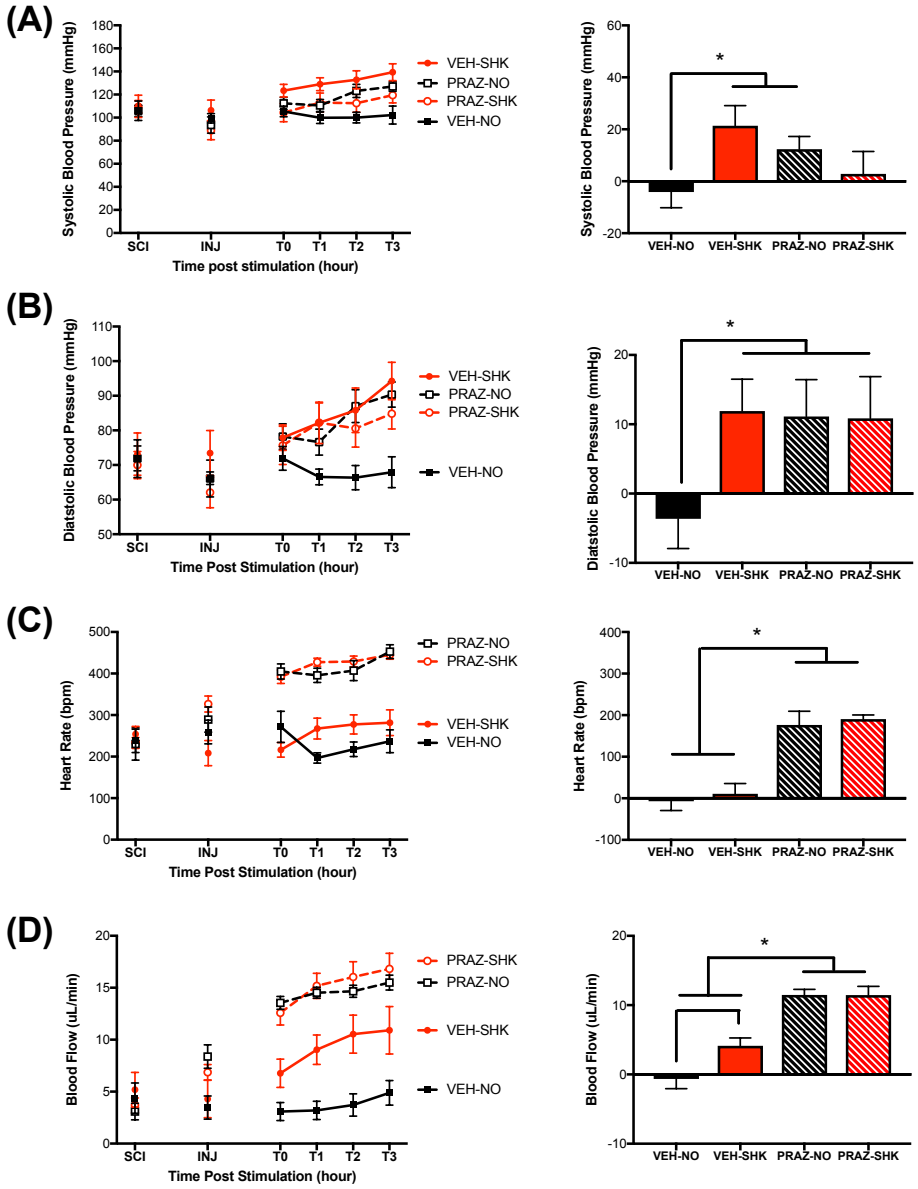


**Figure 35. Blood pressure three hours after prazosin and shock.** Prazosin blocked the increase in systolic blood pressure, but increased diastolic blood pressure, heart rate and blood flow. Shock increased systolic and diastolic blood pressure, heart rate, and blood flow in vehicle treated subjects. Error bars represent SEM (n = 8).

Hypertension was not correlated with hemorrhage in any groups, $r < 0.69$, $p > 0.05$ (data not shown).

Elevated heart rate was correlated with an increase in hemorrhage in shocked subjects receiving prazosin. Using a Pearson's correlation coefficient, I assessed the relationship between heart rate and the absorbance of hemoglobin (420 nm) in tissue collected at three hours. In prazosin subjects given shock, a strong positive correlation was found between heart rate at two and three hours and hemorrhage, $r = 0.76$ and 0.75 (respectively), $p < 0.05$. Heart rate was not correlated with any other groups, $r < 0.62$, $p > 0.05$ (data not shown).

Increased blood flow was correlated with an increase in hemorrhage in unshocked prazosin subjects. Using a Pearson's correlation coefficient, a strong positive correlation was found in unshocked prazosin subjects between blood flow immediately and one hour after stimulation and hemorrhage, $r = 0.82$ and 0.72 (respectively), $p < 0.05$. Blood flow was not correlated with any other groups, $r < 0.49$, $p > 0.05$.

Hemorrhage was not correlated with locomotor performance in any groups, $r < 0.65$, $p > 0.05$ (data not shown).

An increase in hypertension was correlated with a decrease in locomotor performance in unshocked subjects. Using a Pearson's correlation coefficient, I assessed the relationship between blood pressure and locomotor performance. A strong negative correlation was found in unshocked subjects between systolic blood pressure and MAP at two hours with locomotor performance a three hours for vehicle ($r = -0.71$ and -0.73, respectively) and prazosin treated subjects ($r = -0.73$ and -0.72, respectively), $p < 0.05$.

Additionally, unshocked prazosin subjects had a correlation between systolic and diastolic blood pressure and MAP at three hours and locomotor scores at three hours, $r$ = -0.85, -0.94, and -0.93 (respectively), $p < 0.05$. Systolic blood pressure immediately and two hours after stimulation was correlated with locomotor scores at three hours, $r$ = -0.77 and -0.71 (respectively), $p < 0.05$. Finally, there was a correlation between diastolic blood pressure and MAP at three hours and locomotor performance at two hours, $r$ = -0.82 and -0.83 (respectively), $p < 0.05$. Hypertension was not correlated with locomotor performance in shocked subjects, $r < 0.60$, $p > 0.05$ (data not shown).

An increase in heart rate was correlation with a decrease in locomotor performance in prazosin subjects receiving electrical stimulation. Using a Pearson's correlation coefficient, a strong negative correlation was found in shocked subjects given prazosin between heart rate immediately and two hours after shock and locomotor scores at one hour, $r = 0.72$ and 0.75 (respectively), $p < 0.05$. Heart rate was not correlated with any other groups, $r < -0.68$, $p > 0.05$ (data not shown).

An increase in blood flow was correlated with a decrease in locomotor performance in vehicle treated subjects. Using a Pearson's correlation coefficient, a strong negative correlation was found in vehicle unshocked and shocked subjects between blood flow at two hours and locomotor scores at one hour, $r$ = -0.78 and -0.82 (respectively), $p < 0.05$. In unshocked vehicle subjects, there was also a correlation between blood flow immediately and one hour after injection, $r$ = -0.73 and -0.78 (respectively), $p < 0.05$. Blood flow was not correlated with prazosin groups, $r < -0.38$, $p > 0.05$ (data not shown).

*Discussion*

Prazosin blocked the increase in systolic blood pressure seen after electrical stimulation. Further, prazosin blocked the induction of the hemorrhage seen at three hours. This suggests that changes in blood pressure may be important in expanding the hemorrhage after noxious input.

The current study also replicated previous work showing that electrical stimulation increased hemorrhage, blood pressure, and blood flow in vehicle treated subjects.

Unexpectedly, prazosin given to unshocked controls showed a paradoxical increase in systolic and diastolic blood pressure, heart rate and blood flow. This may be either a compensatory mechanism or a rebound effect. Typically prazosin has only been looked at in subjects and patients with higher-level injuries. It could be that prazosin acts differently after a low-level SCI.

Prazosin did not block the decrease in locomotor scores seen after electrical stimulation. This could be partially due to the increase in lethargy seen after prazosin and shock. Within the prazosin-shocked group, 6 out of the 8 rats were noted as lethargic, defined as a failure to move when placed in the open field during BBB. Only 2 out of the 8 rats in the prazosin unshocked group and none of the vehicle treated group showed a similar lethargy. Future studies should examine locomotor scores after the drug effects subside.

## Experiment 12: Blocking the Blood Pressure Increase on Long-Term Recovery

In the previous experiment, prazosin blocked indices of hemorrhage for up to three hours after shock. In this study, I explored whether prazosin treatment could lead to better locomotor recovery at 21 days post injury.

### *Procedure*

Twenty-four hours after receiving a moderate spinal cord contusion at T12, male Sprague Dawley rats (n = 8) had their locomotor scores evaluated using the BBB locomotor scale and their baseline blood pressure measurements taken using a non-invasive blood pressure cuff. Subjects were then randomly assigned to prazosin (3 mg/kg) or vehicle (30% glucose). Groups were balanced using baseline systolic blood pressure and BBB scores. Six minutes after the injection of drug, subjects had their blood pressure monitored. Thirty minutes after drug administration, subjects were randomly assigned to electrical stimulation (180 shocks, 1.5mA, 0.2-3.8 second ISI) to the tail or remained unshocked. BBB scores were monitored on days 1-7, 10, 14, and 21 post injury. Blood pressure was monitored three hours after electrical stimulation in all subjects. In a subset of subjects (n = 4), blood pressure was monitored on day two and seven post-injury to examine how long the blood pressure effected lasted. On day 21, subjects able to weight support were tested on the beam and ladder tasks to evaluate fine motor skills and balance. All subjects were then evaluated for at-level pain using the girdle test. Once all testing was finished, subjects were given a lethal dose of pentobarbital and their tissue perfused and collected for lesion analysis.

Two subjects in the vehicle-shocked group died within the first week (i.e., day five and seven) after injury. They were removed and their data replaced. No mortalities were seen in the other groups.

*Results*

Locomotor scores did not differ between groups prior to drug treatment. BBB scores obtained the day after surgery, prior to treatment, ranged from 3.06 (± 0.20) to 3.31 (± 0.40). An ANOVA revealed no difference between groups, all $F$s < 1.0, $p > 0.05$.

BBB scores were obtained daily from days two to seven and on days 10, 14, and 21 after injury. A repeated measures ANCOVA with stimulation and drug as the between subjects variable, baseline blood flow as a covariate, and time as a repeated measure found a main effect of shock and time and an interaction between shock and time, all $F$s > 4.55, $p < 0.05$ (Figure 36A). Analysis of the interaction between shock and time found significantly lower locomotor scores in subjects that received electrical stimulation. This effect was not blocked by prazosin.

Subjects that were capable of weight support were tested on the beam and ladder tasks: seven subjects in the vehicle unshocked, six in the prazosin unshocked, five in the prazosin shocked, and one in the vehicle shocked group. A chi-squared examining the number of subjects able to wait support found no significance, $p > 0.05$. An ANOVA found no difference between groups, $F > 2.78$, $p < 0.05$ (data not shown).

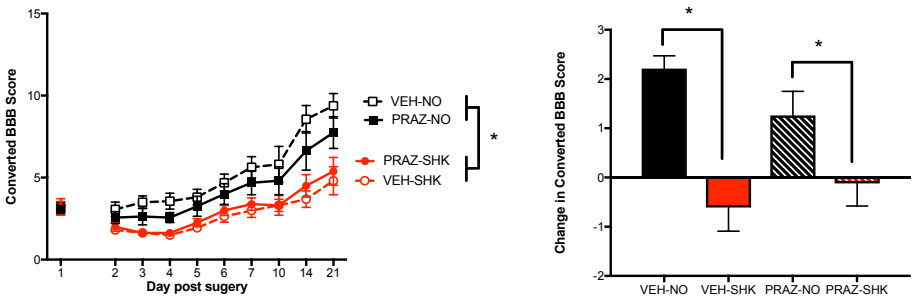


**Figure 36. Long-term locomotor recovery after prazosin and shock.** BBB scores were decreased after electrical stimulation. Error bars represent SEM. (n = 8)

Blood pressure measurements were not different between groups prior to treatment. Pre-treatment values ranged from 102.25 (± 6.86) to 103.37 (± 5.67) mmHg for systolic, 65.54 (± 3.07) to 67.83 (± 3.88) mmHg for diastolic, 77.74 (± 3.49) to 79.39 (± 4.38) mmHg for MAP, 217.74 (± 20.7) to 262.90 (± 30.24) bpm for heart rate, and 3.13 (± 0.33) to 5.44 (± 2.02) μL per minute for blood flow. Independent ANOVAs revealed no difference between groups, all $F$s > 1.75, $p > 0.05$. An injection of prazosin significantly lowered baseline heart rate and blood flow, $F$ (1, 32) > 44.93, $p < 0.001$.

Shock had no effect in prazosin treated animal at the immediate time point. However by day two, prazosin subjected treated with stimulation showed an increase in hypertension. Blood pressure measurements were taken at three hours, two and seven days after stimulation in a subset of animals (n = 4). To examine changes in blood pressure, I used a repeated measures ANCOVA with stimulation and drug as between subject variables, baseline as a covariate, and time as a repeated measure. Omnibus analysis revealed a main effect of shock, a two-way interaction between time and shock, and a three-way interaction between time, drug and shock, $F > 3.57$, $p < 0.001$ (Figure 37A). No other effects were statistically different, $F < 4.82$, $p > 0.05$. *Post hoc* comparison of the three-way interaction found that prazosin treated subjects and vehicle subjects receiving stimulation showed an initially increased in hypertension. On day two post-injury, prazosin unshocked subjects showed a significant drop in blood pressure. Shocked subjects continued to show an increase in blood pressure compared to unshocked subjects. By day seven, the hypertension in shocked groups significantly dropped. There was still a significant different between shocked subjects and vehicle

unshocked subjects. Similar results were seen for diastolic blood pressure and MAP, $F > 4.82$, $p < 0.05$ (data not shown).

Heart rate was elevated in prazosin treated subjects and in vehicle treated subjects that experience shock. Heart rate measurements were taken at three hours, two and seven days after stimulation in a subset of animals (n = 4). To examine changes in heart rate, I used a repeated measures ANCOVA with stimulation and drug as between subject variables, baseline as a covariate, and time as a repeated measure. Omnibus analysis revealed a main effect of drug and an interaction between shock and time, $F > 9.69$, $p < 0.01$ (Figure 37B). No other effects were statistically significant, $F > 3.23$, $p < 0.01$. *Post hoc* comparison of the main effect found an increase in heart rate in subjects treated with prazosin. Analysis of the interaction found that shock only increased the heart rate after electrical stimulation at the three hours time point.

An increase in blood flow was found in prazosin treated subject and in subjects treated with shock. Blood flow measurements were taken at three hours, two and seven days after stimulation in a subset of animals (n = 4). To examine changes in blood flow, I used a repeated measures ANCOVA with stimulation and drug as between subject variables, baseline as a covariate, and time as a repeated measure. Omnibus analysis revealed a main effect of drug and shock, a two-way interaction between time by drug, and a three-way interaction between time, drug, and shock, $F > 5.22$, $p < 0.05$ (Figure 37C). *Post hoc* comparisons found a significant increase in blood flow at three hours post treatment in prazosin subjects and vehicle subjects treated with shock. On day two post-injury, blood flow significantly dropped in prazosin-unshocked subjects. Prazosin

shocked subjects continued to be elevated compared to all other groups. On day seven post-injury, both prazosin and vehicle shocked subjects significantly dropped in blood flow. No groups were statistically different on day seven post-injury.

Weight was not different between groups prior to treatments. Pre-treatment values ranged from 337.75 (± 25.77) to 348.0 (± 8.72) μL. An ANOVA revealed no difference between groups, all $F$s < 1.11, $p > 0.05$.

Weight remained significantly decreased in subjects that received electrical stimulation. Weight was taken daily. To examine the effect of prazosin and shock on weight gain, I used a repeated measures ANCOVA with stimulation as the between subjects variable, baseline weight as a covariate, and time as a repeated measure. Overall analysis found a significant main effect of shock and time, all $Fs > 3.02$, $p < 0.05$. No other effects were statistically significant, all $Fs < 2.08$, $p > 0.05$ (Figure 38). *Post hoc* comparison of the main effect of shock revealed a decrease in weight gain in subjects exposed to electrical stimulation. The main effect of time showed an initial drop in weight gain after injury that slowly rose over time.

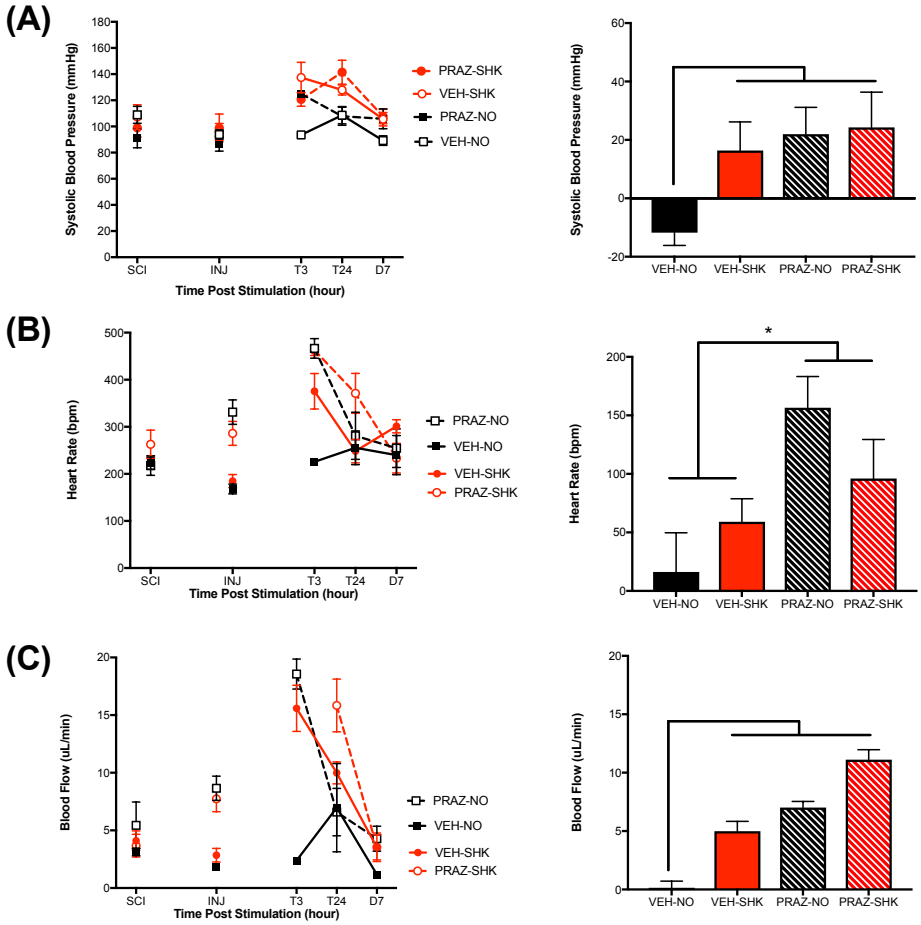


**Figure 37. Blood pressure measurements after prazosin and shock.** Prazosin increased hypertension, heart rate, and blood flow at three hours after stimulation. Electrical stimulation in vehicle treatment subjects increased hypertension, heart rate, and blood flow for up to 24 hours. By seven days heart rate and blood flow returned to baseline measurements in all groups. Error bars represent SEM. (n = 4)

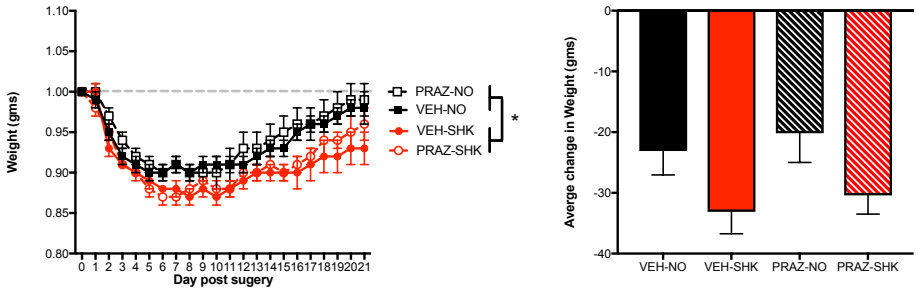


**Figure 38. Change in weight after prazosin and shock.** Electrical stimulation decreased weight gain compared to unshocked controls. SCI caused an initial drop in weight gain after injury that slowly rose over time. Error bars represent SEM. (n = 8)

*Discussion*

In the current study, I replicated our previous results showing that electrical stimulation causes a decreased in weight gain and locomotor recovery in vehicle treated subjects.

Prazosin did not improve long-term recovery. However it is important to note that the data for the vehicle-shocked group is artificially high, as the two subjects that died in this group happened to have the lowest locomotor scores. Additionally, we may see difference if we continued the study out to a later time point given there seems to be a slight separate beginning at day 21 between groups. Further, it might be important to keep a steady state level of prazosin in the system, rather then just a single dose.

Prazosin increased blood pressure values at three hours in vehicle treated subjects, but this returned to normal by six hours. Conversely, prazosin-shocked subjects showed a robust increase for up to a day after stimulation. This increase likely reflected the reduction of the drug in the system. Often when blood pressure medications are lowered or stopped, a rise in blood pressure may occur.

Prazosin did not prevent weight loss although animals did start to gain weight towards the end of the 21-day recovery period. Future studies should examine prazosin at later time points to see if this increase continues after 21 days.

**Experiment 13: Blocking the Blood Pressure Increase on Hemorrhage at 24 Hours**

In Experiment 11, prazosin prevented hemorrhage at three hours after stimulation. However in Experiment 12, there was a rebound in blood pressure in prazosin subjects the day after shock (post injury day two). This could be due to the short half-life prazosin (2-3hours). In this experiment, I examined whether giving two injections of prazosin will prevent this rebound in blood pressure seen in Experiment 12. Further I examined whether the decrease in hemorrhage seen in the Experiment 11 was still present 24 hours after stimulation.

*Procedure*

Twenty-four hours after receiving a moderate spinal cord contusion at T12, male Sprague Dawley rats (n = 6) had their locomotor scores evaluated using the BBB locomotor scale and their baseline blood pressure measurements taken using a non-invasive blood pressure cuff. Subjects were then randomly assigned to receive prazosin (3 mg/kg) or vehicle (30% glucose). Thirty minutes after the first drug administration, subjects were given electrical stimulation (180 shocks, 1.5mA, 0.2-3.8 second ISI) to the tail or remained unshocked. Subject then received a second prazosin or vehicle injection two and a half hours after stimulation. BBB scores and blood pressure were then monitored at three, six, 12, and 24 hours after electrical stimulation. After obtaining the 24 hours post-stimulation BBB score and blood pressure measurement, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue

was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm).

*Results*

Locomotor scores did not differ between groups prior to treatment. BBB scores obtained the day after spinal cord injury, prior to treatment, ranged from 3.17 (± 0.2) to 3.50 (± 0.52). An ANOVA revealed no difference between groups, all $F$s < 1.0, $p > 0.05$.

Nociceptive stimulation decreased locomotor performance. BBB scores were obtained at three, six, 12, and 24 hours after stimulation. A repeated measures ANCOVA with shock and drug as between subjects variables, baseline BBB scores as the covariate, and time as the repeated measure found a main effect of shock, $F > 14.67$, $p < 0.001$. No other effects were statistically significant, all $F$s < 1.59, $p > 0.05$. *Post hoc* comparison of the main effect found a significant decease in locomotor performance after electrical stimulation.

Prazosin did not block the expansion of the hemorrhage 24 hours after stimulation. The magnitude of the peak in absorbance at 420 nm was analyzed using an ANOVA with shock and drug as the between subjects variables. Overall analysis found a main effect of shock and an interaction between shock and drug, $F > 5.17$, $p < 0.05$ (Figure 40). *Post hoc* comparisons found an increase in hemorrhage in subjects receiving prazosin and in vehicle shock subjects, compared to vehicle unshocked controls.

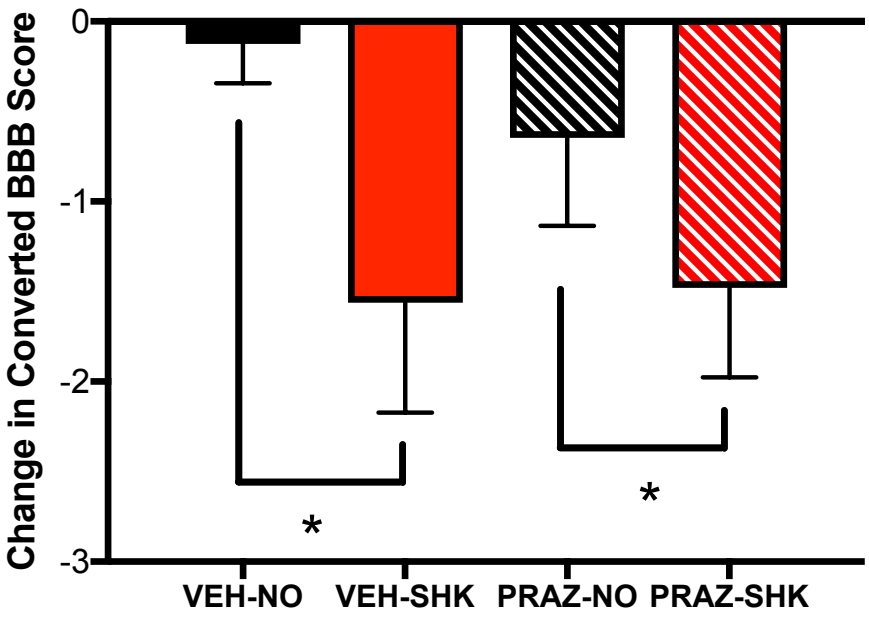


**Figure 39. Locomotor performance 24 hours after prazosin and shock.** Electrical stimulation decreased BBB locomotor scores in both prazosin and vehicle treated subjects. Error bars represent SEM. (n = 6)

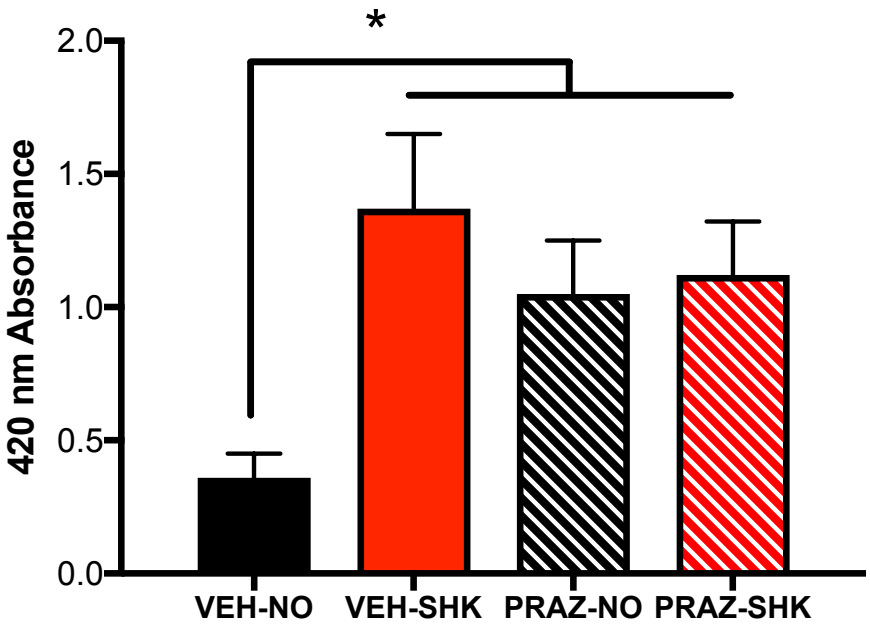


**Figure 40. Hemorrhage 24 hours after prazosin and shock.** The absorbance at 420 nm taken at 24 hours was increased in subjects treated with prazosin and vehicle subjects exposed to electrical stimulation. Error bars represent SEM. (n = 6)

Blood pressure measurements were not different between groups prior to treatment. Pre-treatment values ranged from 111.86 (± 5.36) to 113.77 (± 14.53) mmHg for systolic, 74.26 (± 3.04) to 82.05 (± 5.50) mmHg for diastolic, 86.46 (± 3.49) to 92.18 (± 6.16) mmHg for MAP, 203.49 (± 27.84) to 252.52 (± 14.70) bpm for heart rate, and 3.96 (± 1.26) to 5.22 (± 2.2) μL per minute for blood flow. Independent ANOVAs revealed no difference between groups on any of these measures, all $F$s < 1.0, $p > 0.05$.

Hypertension was produced by prazosin alone and in vehicle treated subjects exposed to shock. Blood pressure measurements were obtained at three, six, 12, and 24 hours after stimulation to examine the effect of stimulation and drug on systolic blood pressure. A repeated measures ANCOVA with stimulation as the between subjects variable, baseline systolic as a covariate, and time as a repeated measure found a main effect of drug and shock and an interaction between drug and shock, $F > 5.10$, $p < 0.05$ (Figure 41A). No other effects were statistically significant, all $F$s < 1.88, $p > 0.05$. *Post hoc* comparison of the main effects showed an increase in hypertension in prazosin subjects compared to vehicle subjects and an increase in shocked subjects compared to unshocked controls. Analysis of the interaction found that hypertension was increased in subjects treated with prazosin and in subjects exposed to shock in the vehicle group. Similar effects were seen for diastolic blood pressure and MAP, $F > 3.20$, $p < 0.05$.

Heart rate was elevated in prazosin subjects and subjects exposed to electrical stimulation. Heart rates were obtained at three, six, 12, and 24 hours after stimulation. To examine the effect of stimulation and drug on heart rate, I used a repeated measures

ANCOVA with stimulation as the between subjects variable, baseline as a covariate, and time as a repeated measure. Overall analysis found a main effect of drug and shock and an interaction between time and drug, $F > 13.07$, $p < 0.001$ (Figure 41B). No other effect were statistically significant, all $F$s $< 1.59$, $p > 0.05$. Analysis of the main effects found elevated heart rate in subjects given prazosin and in subjects that received electrical stimulation. Analysis of the interaction revealed an elevation in heart rate in prazosin subject at three and six hours after treatment compared to vehicle treated subjects.

Blood flow was elevated in prazosin subjects and subjects exposed to electrical stimulation. Blood flow was obtained at three, six, 12, and 24 hours after stimulation. To examine the effect of stimulation and drug on heart rate, I used a repeated measures ANCOVA with stimulation as the between subjects variable, baseline as a covariate, and time as a repeated measure. Overall analysis found a main effect of drug and shock and an interaction between time and drug, $F > 17.98$, $p < 0.001$ (Figure 41C). No other effects were statistically significant, all $F$s $< 2.80$, $p > 0.05$. Analysis of the main effects showed an increase in blood flow in subjects given prazosin and in subjects exposed to shock. Analysis of the interaction between time and drug found that the increase in blood flow for prazosin subjects was significantly different then vehicle treated subjects at three, six and 12 hours after treatment.

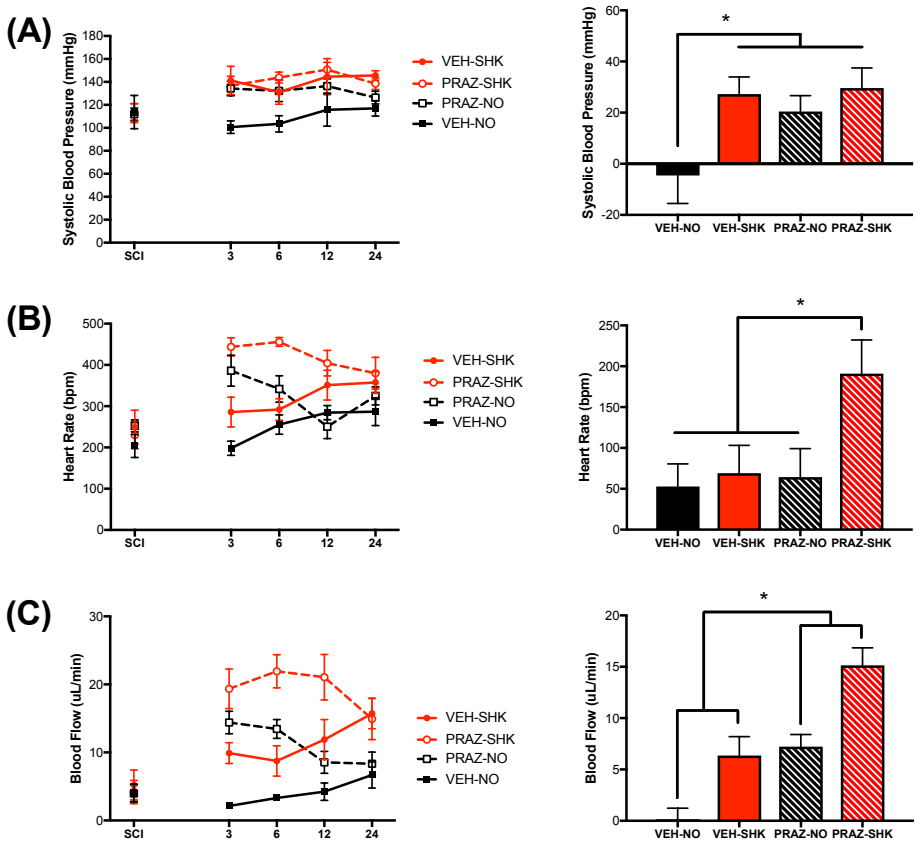


**Figure 41. Blood measure 24 hours after prazosin and shock.** Prazosin increased hypertension, heart rate, and blood flow. In addition, subjects treated with shock also showed an increase in hypertension, heart rate, and blood flow. Error bars represent SEM. (n = 6)

*Discussion*

The current study again replicated earlier experiments showing that subjects treated with vehicle show an increase in hypertension, heart rate, and blood flow after electrical stimulation. Additionally, this study replicated the decrease in locomotor performance and increase in hemorrhage seen after shock in vehicle subjects.

Prazosin did not block the expansion of the hemorrhage 24 hours after stimulation. Prazosin did however block the shock effect, as there was not an additional increase in hemorrhage in prazosin-shocked subjects compared to prazosin-vehicle subjects.

Giving two doses of prazosin three hours apart did not improve blood pressure. Instead prazosin treated subjects that received shock showed higher levels of hypertension, elevated heart rates and increased blood flow compared to vehicle shocked subjects. Additionally, prazosin alone continued to increased hypertension, heart rate, and blood flow for up to six hours. Future studies should explore additional doses or alternative delivery methods. For example, prazosin has been shown to manage hypertension in spontaneous hypertensive rats when given daily in their water. Prazosin may also need to be paired with drugs that help to maintain heart rate within a normal range during treatment.

## Experiment 14: Effect of Pharmacologically Increasing the Blood Pressure

In the current study, I examined the effect of pharmacologically increasing blood pressure with norepinephrine, a nonselective adrenergic agonist, on blood pressure, hemorrhage and locomotor performance.

### *Procedure*

Twenty-four hours after receiving a moderate spinal cord contusion at T12, male Sprague Dawley rats (n = 8) had their locomotor scores evaluated using the BBB locomotor scale and their baseline blood pressure measurements taken using a non-invasive blood pressure cuff. Rats were then randomly assigned to norepinephrine (1 mg/kg) or vehicle (saline). Groups were balanced using baseline systolic blood pressure and BBB scores. BBB scores and blood pressure were monitored immediately, one, two, and three hours after injection. After obtaining the three hours BBB score and blood pressure measurement, subjects were sacrificed and a one-centimeter of spinal cord tissue centered on the lesion was collected and flash frozen. Spinal cords were kept at -80°C until processed. The spinal cord tissue was homogenized and protein extracted. Protein extracts were analyzed for signs of hemorrhage using spectrophotometry (spectral analysis at 420 nm).

### *Results*

Locomotor scores did not differ between groups prior to drug treatment. BBB scores obtained the day after surgery, prior to treatment, ranged from 3.00 (±0.39) to 3.56 (±0.42). An ANOVA revealed no differences between groups, all $F$s < 1.0, $p$ > 0.05.

Norepinephrine had no effect on locomotor scores. A repeated measures ANCOVA with drug as the between subjects variable, baseline as the covariate and time as the repeated measure found no statistically significant effects, $F < 2.14$, $p > 0.05$ (Figure 42).

Norepinephrine did not cause an expansion of the hemorrhage. The magnitude of the peak in absorbance at 420 nm was analyzed using an ANOVA with drug as the between subjects variable. Overall analysis revealed no significant effects, $F(1, 14) < 1.0$, $p > 0.05$ (Figure 43).

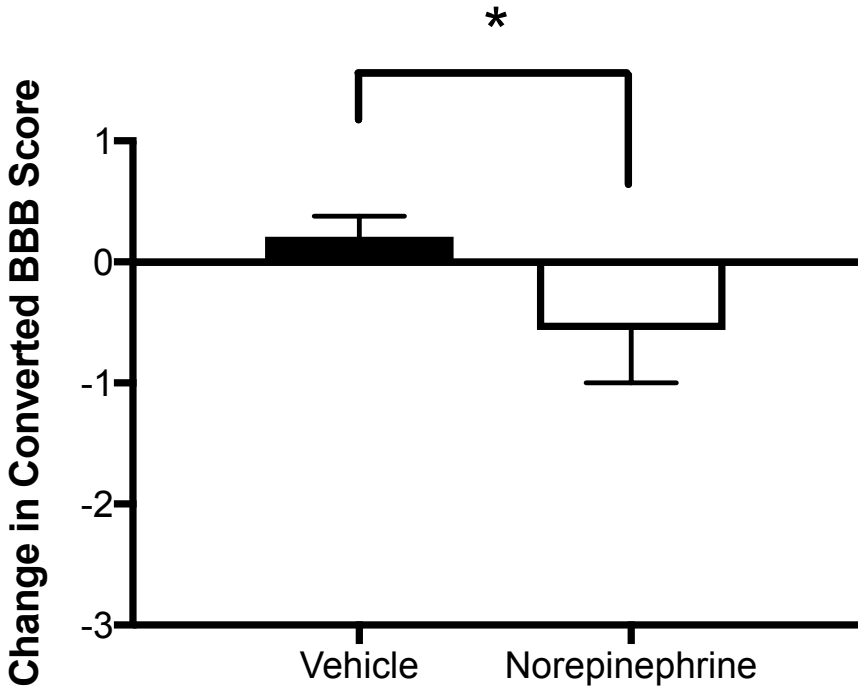


**Figure 42. Locomotor performance over the three hours after norepinephrine.** Norepinephrine decreased locomotor scores over the three hours after injection.

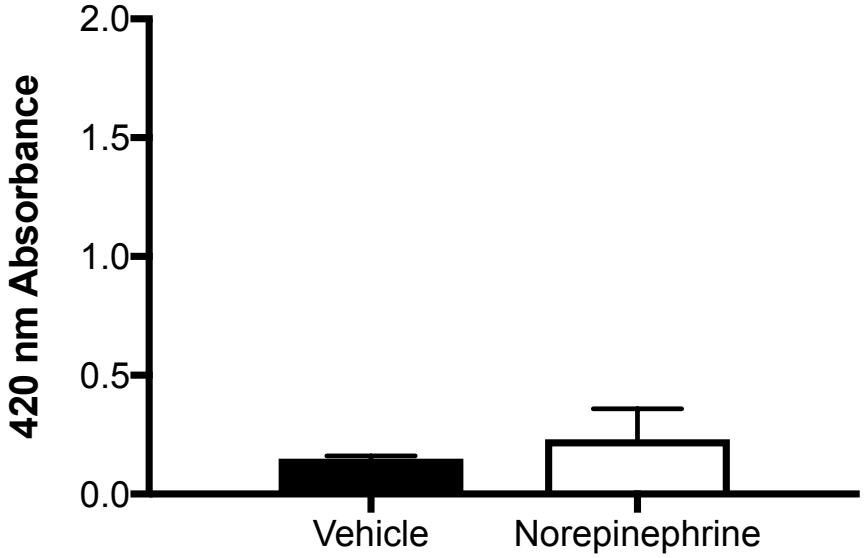


**Figure 43. Hemorrhage three hours after norepinephrine.** The absorbance at 420 nm taken at three hours after injection was not different between norepinephrine and vehicle groups. Error bars represent SEM. (n = 8)

Blood flow was significantly different between groups prior to drug. Pre-treatment values ranged from 112.17 (± 8.35) to 123.61 (± 7.55) mmHg for systolic, 73.27 (± 5.27) to 81.60 (± 6.13) mmHg for diastolic, 85.91 (± 6.18) to 95.23 (± 6.45) mmHg for MAP, 233.31 (± 29.46) to 273.57 (± 26.04) bpm for heart rate, and 3.94 (± 1.05) to 8.13 (± 1.46) μL per minute for blood flow. Independent ANOVA revealed a significant difference between groups for blood flow, $F$ (1, 14) = 5.44, $p > 0.05$. No other effects were statistically significant, $F < 1.15$, $p > 0.05$. *Post hoc* comparison showed that blood flow was increased in subjects placed in the norepinephrine group.

Norepinephrine significantly increased systolic blood pressure. Blood pressure measurements were obtained immediately, one, two and three hours after injection. Using a repeated measures ANCOVA with drug as the between subjects variable, baseline as the covariate, and time as the repeated measure, I found a main effect of main effect of condition, $F$ (1, 13) = 26.48, $p > 0.001$ (Figure 44A). No other effects were statistically significant, $F < 1.0$, $p > 0.05$. *Post hoc* comparison of the main effect showed an increase in systolic blood pressure in subjects treated with norepinephrine. Identical results were seen for diastolic blood pressure and MAP, main effect of condition, $F$ (1, 13) > 22.697, $p > 0.001$. No other effects were statistically significant, $F < 1.0$, $p > 0.05$.

Norepinephrine had no effect on heart rate. Heart rates were obtained immediately, one, two and three hours after injection. Using a repeated measures ANCOVA with drug as the between subjects variable, baseline as the covariate, and time as the repeated measure, I found no significant effects, $F < 2.48$, $p > 0.05$ (Figure 44B).

Blood flow was increased in subjects receiving norepinephrine. Blood flow was obtained immediately, one, two and three hours after injection. Using a repeated measures ANCOVA with drug as the between subjects variable, baseline as the covariate, and time as the repeated measure, I found a main effect of condition, $F$ (1, 13) = 14.78, $p > 0.001$ (Figure 44C). No other effects were statistically significant, $F < 1.0$, $p > 0.05$. *Post hoc* comparison of the main effect revealed an increase in blood flow after norepinephrine treatment.

*Discussion*

Much like electrical stimulation, an injection of norepinephrine increased systolic and diastolic blood pressure, as well as blood flow. Similarly, norepinephrine did not affect heart rate. Norepinephrine also caused a decrease in locomotor performance. This adds to correlative studies showing that blood pressure changes around the time of injury are related to late locomotor recovery (Nielson et al., 2015). Further, the current study points to a causal relationship between increases in blood pressure and decreases in locomotor performance.

Interestingly, an injection of norepinephrine did not cause an increase in hemorrhage at three hours post injection. It may be that the timeline for the development of hemorrhage was different for norepinephrine. This was seen for shock and capsaicin, which show peaks at three and 24 hours, respectively. Therefore it would be important to explore additional time points after norepinephrine. Another possibility could be that blood pressure interacts with process below the injury to cause hemorrhage. For example, local release of substance p and inflammatory cytokines after electrical

stimulation may play a role in hemorrhage development. This would help explain why prazosin was ineffective at blocking the long-term effects of shock treatment.

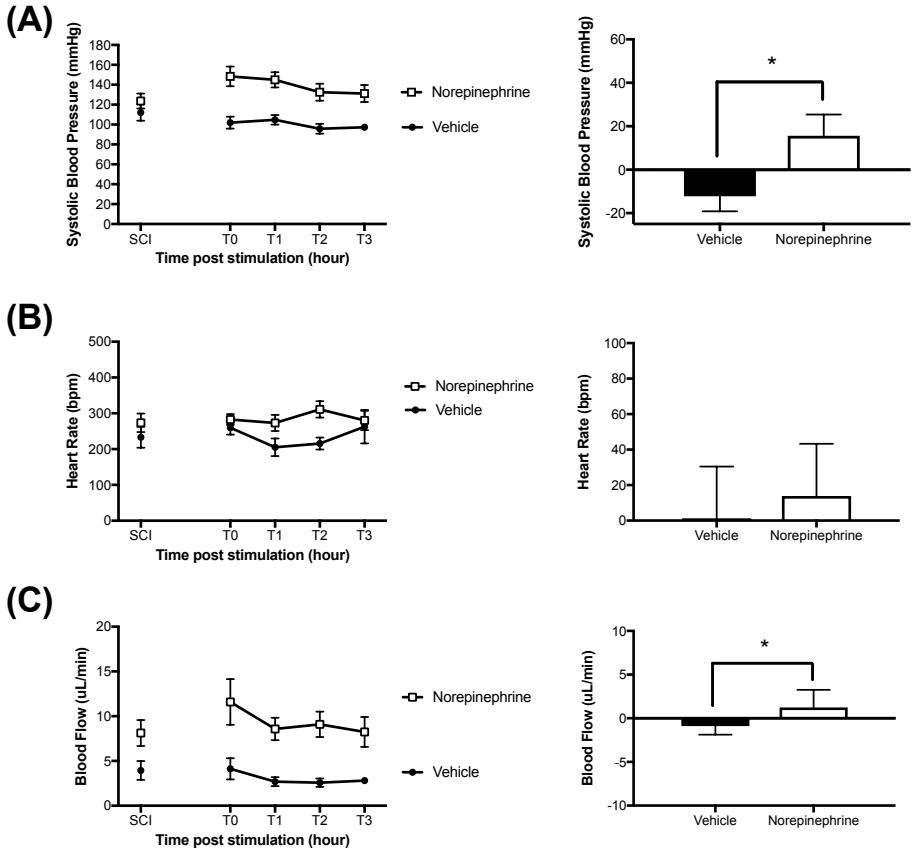


**Figure 44. Blood pressure three hours after norepinephrine.** Treatment with norepinephrine significantly increased systolic blood pressure and blood flow. Error bars represent SEM. (n = 8)

# CHAPTER IX

# GENERAL DISCUSSION AND SUMMARY

In the current dissertation, I examined the conditions under which noxious input initiates hemorrhage after SCI. I also determined the role of blood pressure in producing these effects. I began in Chapter III by examining the time course and stimulation parameters that underlie the induction of the hemorrhage. Then in chapter IV and V, I determined whether noxious input (i.e., electrical stimulation and capsaicin) induced blood pressure changes. In Chapter VI, I determined whether having the ability to control noxious stimulation affected blood pressure and hemorrhage. Next, in Chapter VII, I determined whether there were age and sex differences in hemorrhage and blood pressure after noxious stimulation. Finally in Chapter VIII, I examined whether blood pressure was sufficient and necessary for the expansion of the hemorrhage.

I found that pain input after SCI leads to the expansion of the hemorrhage. Pain input is most damaging soon after SCI and shows a linear relationship (i.e., increasing pain input causes an increase in hemorrhage).

Evaluation of blood pressure after electrical stimulation revealed an increase in hypertension and blood flow. No differences were found after capsaicin treatment. Additionally, sex differences were seen in animals exposed to electrical shock, but not capsaicin. Examination of the impact of controllability of noxious input on hemorrhage, blood pressure and locomotor performance showed that only uncontrollable stimulation induces hypertension.

Finally, treatment of blood pressure with prazosin was found to decrease indices of hemorrhage at three hours. This drug treatment did not, however, block the effect of nociceptive stimulation on locomotor performance. Examination of the long-term effects of prazosin found no protection from the damaging effect of stimulation within 21 days after injury. While promising, further work is needed to better understand how to manage blood pressure after pain input in people with SCI.

## Pain Input Increases Indices of Hemorrhage

Prior work using a contusion model found that six minutes of nociceptive stimulation given a day after a SCI increases tissue loss at the site of injury (Grau et al., 2004; Turtle et al., 2017). Additionally, animals showed decreased locomotor scores, decreased weight gain and increased mortality across the 42-day recovery period (Grau et al., 2004; Turtle et al., 2017). Subsequent research has shown that nociceptive stimulation also promotes acute hemorrhage at the site of injury.

### *Vulnerability of the Spinal Cord*

Examination of the stimulation parameters that initiate hemorrhage showed that stimulation soon after to injury led to more hemorrhage relative to stimulation given days or weeks later. This expands a previous study that showed nociceptive stimulation soon after injury had the greatest impact on long-term recovery (Grau et al., 2004). Taken together, these data indicate that nociceptive stimulation has a greater effect on hemorrhage and long-term recovery when it occurs soon after injury.

Presumably, pain input that co-occurs with injury should induce the greatest hemorrhage. Evidence for this can be seen in studies examining mortality and recovery

in humans, which have shown that secondary trauma is associated with higher rates of mortality and a decrease in neurological recovery (Tator, Duncan, Edmonds, Lapczak, & Andrews, 1995). While this may be partially related to the level of injury (i.e., thoracic injuries tend to have more associated injuries), one study controlling for this relationship still found a correlation between associated trauma and neurological recovery (Tator et al., 1995).

### *Linear Relationship Between Pain and Hemorrhage*

The parameters used to study the effect of nociceptive stimulation (six minutes of intermittent shock given at an intensity of 1.5 mA) were derived from studies exploring the development of nociceptive sensitization and maladaptive plasticity after a complete spinal transection (Baumbauer et al., 2008). Here I examined whether less intense and/or shorter durations of stimulation affect the development of hemorrhage in contused rats. I found that less intense and shorter durations of shock stimulation induced hemorrhage. This work indicates a linear relationship between nociceptive input and hemorrhage and suggests that even small amounts of pain change locomotor outcomes.

The linear relationship between pain and hemorrhage is likely the results of increased C-fiber activation and neurotransmitter release (glutamate and substance P). These signals can trigger the activation of cell death pathways and PHN. Additionally, the increase in hemorrhage could be due to graded changes in blood pressure, with more stimulation causing more hypertension. This increase in blood pressure could then cause a breakdown of the capillaries, resulting in red blood cell infiltration into the spinal

tissue. Because treatment of SCI can engage nociceptive fibers, further work is needed to determine the intensity/duration values that are safe and do no long-term harm.

### *Sex Difference in Hemorrhage*

Previous work in animals has observed better functional outcomes in females compared to males after SCI (Datto, Bastidas, et al., 2015; Datto, Yang, et al., 2015). In particular, females tend to show more tissue sparing compared to males with similar injuries.

Both shock and capsaicin produced an increase in hemorrhage and a decrease in locomotor performance in males. In females, only capsaicin produced an increase in hemorrhage and a decrease in locomotor performance.

The lack of hemorrhage expansion in females exposed to electrical stimulation points to a difference in susceptibility to hemorrhage between males and females. This difference could be due to a difference in pain tolerance. It could be that the electrical stimulation used in this study was not strong enough to induce a hemorrhage. Evidence of this difference was seen during treatment where it was casually noted that females habituated to the stimulation within a few minutes, while males continued to react across the full six minutes. Given that females showed a difference to injected capsaicin, indicates that they are sensitive to pain, although the lack of hemorrhage in shock indicates that they may not be as sensitive to hemorrhage expansion.

Both sexes reacted similarly after capsaicin injection. This could mean that the effect of capsaicin injection is the same across sexes or the ability to see differences between sexes is masked by a ceiling effect of capsaicin on hemorrhage. Further, work is

needed to compare the effect of capsaicin across a range of dose.

The time course for hemorrhage and locomotor deficits were different for shock and capsaicin. For shock, expansion of the hemorrhage and decreased locomotor performance were only seen at three hours. In capsaicin, these effects were observed at both three and 24 hours after injection. This replicates prior work (Turtle et al., 2017), and suggests that pain input is associated with an expansion of hemorrhage. It also points to a difference between the two models of pain input. Electrical stimulation was given for only six minutes and was not associated with inflammation or tissue damage at the site of stimulation. Capsaicin produces a long lasting pain that inflames the surrounding tissue. The difference in the amount and duration of hemorrhage may be due to the difference in the duration of activation of pain fibers. However, it could also reflect differences in inflammation.

Future studies will need to examine the stimulation parameters necessary for hemorrhage expansion in females. Further, because an injection per se has an affect in male rates, it is recommended that future studies apply capsaicin topically. Additionally, long-term analysis of females after pain input should be done to see if the observed changes in hemorrhage are sufficient to cause a deficit in recovery and tissue sparing. Future studies should also examine whether co-application of inflammatory cytokines and stimulation would mimic the longer hemorrhage duration produced by capsaicin.

### *Hemorrhage is Correlated with Locomotor Performance*

Previous studies have shown that hemorrhage impacts locomotor recovery (Simard et al., 2007; Turtle et al., 2017). In the current study, a similar relationship was

found with both models of nociceptive input. For electrical stimulation, hemorrhage at 24 hours was correlated with locomotor performance in males. For females who received electrical stimulation and males who received capsaicin stimulation, locomotor performance and hemorrhage were correlated at three hours. There was only a trend towards replicating these results in males in Experiment 10. For females that received capsaicin there was no significant relationship between locomotor performance and hemorrhage. These results point to a complicated relationship between early indices of hemorrhage and locomotor performance. More work is needed to determine the directionality of these relationships.

Results from the above experiments suggest that pain input can increase the extent of the hemorrhage, which would expand the region of secondary injury. This can then lead to an increase in lesion volume and a reduction in locomotor performance. One mechanism involved in hemorrhage expansion is the formation of the SUR1-TRPM4 complex. This complex is formed in response to depleted intracellular ATP and leads to cell depolarization, cytotoxic edema, and cell death (Simard et al., 2007). Research has shown that blocking the formation of this channel with glibenclamide after SCI, prevents the capillary fragmentation and red blood cell infiltration, leading to better locomotor recovery and tissue sparing (Simard et al., 2007).

One treatment that has been found to prevent the effect of nociceptive stimulation on hemorrhage and locomotor recovery is lidocaine (Turtle et al., 2017). Lidocaine is a sodium channel blocker that prevents the propagation of action potentials. Given that pain signals at the site of injury appear to play a critical role in activating

PHN and creating an excitotoxic environment, lidocaine was administered locally to block these signals. Blocking the barrage of signals before pain input blocks the induction of the hemorrhage, prevents the expansion of the lesion, and improves long-term recovery. These studies suggest a strong relationship between hemorrhage and recovery. Examination of the mechanisms by which hemorrhage expansion occurs will be important for finding new treatments.

### Nociceptive Input Impacts Blood Pressure

Blood pressure after SCI is one of the best predictors of locomotor recovery in rats and humans (Kepler, Schroeder, et al., 2015; Nielson et al., 2015). Given research showing that nociceptive stimulation causes diminished recovery, this dissertation sought to examine whether blood pressure played a role in these effects. Using two forms of nociceptive input, I found differences in how they interact with the cardiovascular system. For animals that received electrical stimulation, an increase in hypertension, heart rate, and blood flow were observed for up to three hours in both males and females. Further, the increase in blood flow was found to last up to 24 hours in males. This indicates an activation of the cardiovascular system by electrical stimulation. For animals injected with capsaicin, no significant effects on blood pressure were observed. While there was a transient increase in blood flow in male rats in Experiment 6, this did not replicate in Experiment 9. Previous research has shown that capsaicin produces an inconsistent triphasic effect on blood pressure that is dose-dependent (Chahl & Lynch, 1987). Therefore, it could be that capsaicin causes a transient spike and depressions in blood pressure that was not captured here. Further, the

inconsistent nature of capsaicin on blood pressure could have masked the effects on blood pressure. For example, if some animals react to capsaicin with a decrease in blood pressure, while others show an increase, the group data would show no difference.

### *Hypertension is Correlated with Hemorrhage*

Previous work has shown that inducing hypertension (180 mmHg) after SCI produces an increase in hyperemia (Guha et al., 1989). Similarly, hypertension has been shown to increases hemorrhage and blood brain barrier permeability in models of TBI and stroke (Hardebo & Beley, 1984; Ito et al., 1980).

In this dissertation, I found a similar relationship between blood pressure and hemorrhage in males that received electrical stimulation. In particular, an increase in hypertension and blood flow was correlated with an increase in hemorrhage at three and 24 hours after electrical stimulation. This was seen in both experiments examining males after stimulation, although the correlation was only a trend in Experiment 9. This could indicate that inducing an increase in blood pressure can lead to changes in hemorrhage. Conversely, it could be that hemorrhage affects blood pressure. More work would need to be done to determine the directionality of these effects.

For females exposed to shock and capsaicin and males exposed to capsaicin, no reliable correlations between blood pressure and hemorrhage were observed. While there was an increase in heart rate in capsaicin treated males in Experiment 7, this did not replicate in Experiment 10. It is not surprising that there were no correlations in these groups as no difference in either hemorrhage or blood pressure were observed.

*Hypertension is Correlated with Locomotor Performance*

As previously reported, I found a correlation between changes in blood pressure and locomotor performance (Kepler, Kong, et al., 2015; Nielson et al., 2015; Tator et al., 1995). This relationship was different for the two pain models.

For both males and females that received stimulation, an increase in hypertension and blood flow was correlated with a decrease in locomotor performance. This indicates a negative relationship between blood pressure and performance. Conversely, increases in blood pressure correlated with improvements in locomotor performance after capsaicin injection. For capsaicin treated males, an increase in heart rate and blood flow was correlated with an increase in locomotor scores. For females, an increase in hypertension was correlated with an increase in locomotor performance. These results indicate a positive relationship between blood pressure and motor performance.

While these two results seem contradictory, they may actually represent two sides of a spectrum. Research has shown that both hypertension and hypotension can adversely affect neural injury. Hypertension likely puts a strain on the vascular system leading to a break down of the capillaries, while hypotension reduces perfusion of tissue leading to an increase in ischemia. Thus for shock, hemorrhage is likely the result of hypertension breaking down blood vessels. For capsaicin, the increase in hemorrhage is likely the results of ischemia caused by hypotension.

There are a number of theories as to how blood pressure could affect locomotor performance. We know from our previous work and prior studies that hemorrhage contributes to secondary injury and impacts recovery (Simard et al., 2007; Turtle et al.,

2017). Others have shown that changes in blood pressure are related to a break down of the blood spinal cord barrier, an increase in pro-inflammatory cytokines, and an increase in spinal cord edema (Nielson et al., 2015). This work stresses the importance of maintaining blood pressure within a normal range after SCI. In the case of capsaicin, it also provides evidence that pain input interacts with blood pressure even when the blood pressure is not significantly elevated or reduced. Long-term studies examining how changes in blood pressure and locomotor recovery covary would help shed light on the directionality of these effects.

It should be noted that the correlations for males in both shock and capsaicin seen in Experiments 5 and 7 did not replicate in Experiment 9 and 10, respectively. This may be tied to the increase in variability observed in the latter studies, which may be related to the use of younger/lighter rats (370 vs. 320 grams).

### Control Over Stimulation Attenuates Blood Pressure

Motivated by past work demonstrating that behavioral control can mitigate the adverse effects of nociceptive stimulation (Grau et al., 2004), I compared the effect of controllable and uncontrollable stimulation on blood pressure and hemorrhage. I found that rats given nociceptive stimulation in a controllable manner (Master) exhibited less hypertension, relative to the rats that received uncontrollable stimulation. Controllable stimulation also failed to induce hemorrhage and had no acute effect on locomotor performance. This is consistent with prior studies demonstrating the controllable stimulation fosters adaptive plasticity and does not impair locomotion (Grau et al., 1998; Grau et al., 2004). These results are important as electrical stimulation is often employed

after SCI in humans (Ragnarsson, 2008). This stimulation is usually given as a continuous stream of electrical pulses. While I did not examine the effect of continuous stimulation on hemorrhage and blood pressure in the current study, it has been noted that continuous electrical stimulation after SCI can cause episodes of autonomic dysreflexia in patients with injuries above T6 (Ashley et al., 1993). If possible, clinicians and researcher should try presenting stimulation in a controllable manner to avoid inducing hypertension.

Replicating previous studies (Grau et al., 2004), stimulation given in an uncontrollable manner caused an increase in blood pressure. However, the increase exhibited by Yoked controls was small compared to the increase produced by noxious stimulation to the tail. Further, these animals did not show the increase in hemorrhage and decrease in locomotor performance observed in earlier experiments. A number of differences could account for these disparities. First, stimulation was given through the leg for learning. In all other studies, stimulation was given to the tail. However, this is unlikely to account for these differences as previous work has shown that stimulation to the tail or leg produce similar effects on spinal cord plasticity and pain reactivity after SCI (Baumbauer et al., 2012; Crown et al., 2002). Another potentially important difference is that the stimulation intensity was lower for leg stimulation compared to tail shock (0.6 mA and 1.5 mA, respectively). This could play a role as we have shown that intensity is linearly correlated with hemorrhage and locomotion. However, 0.6 mA is within the range we found to increase hemorrhage after leg shock. The density of stimulations is also different between studies. Yoked rats received an average of 146

shocks across the 30 minutes, with an average of 52 stimuli within the first six minutes. Other studies gave 180 shocks within six minutes. The combination of fewer shocks with a lower intensity would be expected to lessen the impact of nociceptive stimulation on injury. Finally, in the previous study examining the effect of controllability on locomotor performance, two days of training was used (Grau et al., 2004). In contrast, just one day of training was used here. In the previous study, lasting changes in locomotor recovery and lesion volume were observed, whereas no differences were observed in hemorrhage and locomotor performance. This again suggests that more training may be required for these effects to emerge.

**Manipulating Blood Pressure**

Prior work has found a relationship between blood pressure, hemorrhage, and locomotor performance (Guha et al., 1989; Nielson et al., 2015). However, few have experimentally manipulated blood pressure. Here I found that reducing the increase in systolic blood pressure with prazosin before shock produced a decrease in the amount of hemorrhage at three hours, but not 24 hours. This suggests that blood pressure plays a role in the early expansion of the hemorrhage. Clinicians should aim to normalize blood pressure soon after injury in order to help prevent hemorrhage expansion.

Prazosin given before shock did not improve long-term locomotor recovery. At early time points drug-induced locomotor deficits were likely due to an increase in lethargy, which is a side effect of prazosin. However, at later time points, prazosin failed to improve locomotor performance above shock alone. It is important to note that two subjects were removed and replaced in the shock group. If these subjects are included in

the analysis and the remaining BBB scores turned to zero, there was a significant differences between prazosin shock and shock alone. Also, we may not have waited long enough to observe an effect on locomotion. Given that prazosin shocked subjects appeared to exhibit a late improvement in locomotor abilities at 21 days, it would be important for future studies to expand the recovery period.

One unexpected outcome of prazosin treatment was the increase in blood pressure in subjects treated with prazosin alone. Prazosin works by blocking alpha-1 adrenoreceptor in the periphery, causing vasodilation of blood vessels. Examination of prazosin in sham controls may help to elucidate whether this effect is unique to SCI.

Another unexpected consequence of prazosin that may play a role in the hemorrhage and decrease in locomotor scores seen 24 hours after simulation was the rebound in blood pressure the day following treatment. I tried blocking this increase by providing two doses of prazosin spaced three hours apart, but this did not improve locomotion or hemorrhage. A more aggressive and longer lasting regimen may be necessary to prevent these detrimental effects and promote recovery.

Finally, I found that inducing an increase in blood pressure similar to that produced by electrical stimulation using norepinephrine, undermined locomotor performance. However, norepinephrine did not affect hemorrhage. This lack of a hemorrhage effect suggests that blood pressure is not sufficient to produce a hemorrhage. More work is needed to understand the initiating mechanism of hemorrhage and how this relates to blood pressure. Caution is also needed regarding the interpretation of the drug effect on locomotor performance because norepinephrine

generally reduced behavioral activity.

## Future Directions

A potential limitation of this study was that we applied nociceptive simulation prior to the assessment of blood pressure in a different context. This was done, in part, to reduce the contribution of conditioned fear. Further, time was needed to set the animals up for assessment of blood pressure and to acclimate them to the chambers. As a result, the first blood pressure values were not obtained until 15 minutes after treatment (6 minutes of treatment plus 10 minutes of acclimation). This means that we may have missed early changes in blood pressure. Future studies should use implantable devices to obtain readings during stimulation. This will also allow us to monitor blood pressure and heart rate after animals are removed from the restraining tubes and factors that might contribute to the generalization of fear.

In this study, I only studied one mechanism of blocking blood pressure. It would be important to examine other methods of blocking hypertension in order to determine whether the spike in blood pressure is mediated through a particular pathway. I chose the alpha-1 adrenoreceptor blocker, prazosin, because it was shown in a controlled study to prevent hypertension in people with SCI (Krum et al., 1992). Additionally, other drugs within the same class have also showed promise in treating hypertension (Vaidyanathan et al., 1998).

Other methods of controlling blood pressure could be beneficial in preventing the spike induced by noxious stimulation. Most of the current treatments prescribed to people with SCI work in the periphery. For example, nitrates, the most commonly

prescribed agent for treating acute episodes of hypertension after SCI, cause a relaxation of vascular smooth muscle through the release of nitric oxide. Similarly, nifedipine reduces peripheral resistance by blocking the calcium influx to vascular cells. Finally, captopril acts peripherally to dilate arteries by preventing the conversion of angiotensin I to angiotensin II. These drugs, along with prazosin, are the most commonly used drugs for the treatment of hypertension after SCI. Other methods used in the general population are beta-blockers, diuretics, and alpha-2 antagonists.

It will be important to examine whether decreasing the blood pressure regardless of mechanism reduces hemorrhage or whether the mechanism matters at all. If only one type of drug works to reduce hemorrhage, this would help clarify the processes involved. If all types of drugs decrease hemorrhage, then it would mean increases in blood pressure itself leads to hemorrhage. Further, a combination of drugs aimed at blocking both peripheral and central mechanism of blood pressure control may prove even more beneficial after SCI.

A long-term study of the impact of pain input in females is needed in order to determine whether a protective effect is at play. However, before this can be done, a parametric study is needed. Parametric studies are also needed to examine the effects of capsaicin after SCI.

## Implications

The findings point to the importance of managing blood pressure after pain input. Maintenance of blood pressure within the normal range should be the goal. It also indicates that early hemorrhage affects long-term recovery. Thus treatments such as

lidocaine should be employed soon after injury in order to prevent this expansion. Prazosin may also help to reduce early hemorrhage, however, it may need to be combined with other drugs or given over a longer period of time. Morphine should be avoided, as it has been previously shown to cause more damage after SCI (Hook et al., 2007).

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